Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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

NAME

ARCHITECT Homocysteine

INTENDED USE

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 iSystem. Homocysteine values can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.

SUMMARY AND EXPLANATION OF THE TEST

Homocysteine (HCY) is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Homocysteine is exported into plasma where it circulates, mostly in its oxidized form, bound to plasma proteins as a protein-HCY mixed disulfide with albumin.[1-4] Smaller amounts of reduced homocysteine and the disulfide homocystine (HCY-SS-HCY) are present. Total homocysteine (tHCY) represents the sum of all homocysteine species found in serum or plasma (free plus protein bound).

Homocysteine is metabolized to either cysteine or methionine. In the vitamin B6 dependent trans-sulphuration pathway, homocysteine is irreversibly catabolized to cysteine. A major part of homocysteine is remethylated to methionine, mainly by the folate and cobalamin dependent enzyme methionine synthase. Homocysteine accumulates and is excreted into the blood when these reactions are impaired.[2, 4] Impaired homocysteine metabolism results in hyperhomocysteinemia (increased levels of homocysteine in plasma or serum) or homocystinuria (high plasma levels cause homocysteine to be excreted in urine). Hyperhomocysteinemia is caused by nutritional and genetic deficiencies. The majority of elevated homocysteine cases (two-thirds) in the general population are due to deficiency of folic acid, vitamin B6 and vitamin B12.[5]

Severely elevated concentrations of total homocysteine are found in subjects with homocystinuria, a rare genetic disorder of the enzymes involved in the metabolism of homocysteine. Patients with homocystinuria exhibit mental retardation, early arteriosclerosis and arterial and venous thromboembolism.[1, 6] Other less severe genetic defects which lead to moderately elevated levels of total homocysteine are also found.[7-9]

Studies have investigated the relationship between elevated homocysteine concentrations and cardiovascular disease (CVD), indicating homocysteine as an important marker for risk assessment. In the presence of known coronary artery disease (CAD), it has been shown to be a strong independent marker of subsequent CAD-related death.[10] A study conducted on 1933 elderly men and women from the Framingham Heart Study cohort demonstrated that elevated levels of homocysteine are independently associated with increased rates of all-cause and cardiovascular disease mortality.[11] In intermediate risk patients, elevated homocysteine levels are associated with the quantity of coronary artery calcification. Elevated homocysteine levels in these patients are independent of coronary heart disease (CHD) risk factors.[12]

A meta-analysis of 27 epidemiological studies, including more than 4000 patients, estimated that a 5 µmol/L increase in total homocysteine was associated with an odds ratio for CAD of 1.6 (95% confidence interval [CI], 1.4 to 1.7) for men and 1.8 (95% CI, 1.3 to 1.9) for women; the odds ratio for cerebrovascular disease was 1.5 (95% CI, 1.3 to 1.9). The risk associated with a 5 µmol/L increase in total homocysteine was the same as that associated with 0.5 mmol/L (20 mg/dL) increase in cholesterol. Peripheral arterial disease also showed a strong association.[13]

Patients with chronic renal disease experience an excess morbidity and mortality due to arteriosclerotic CVD. An elevated concentration of total homocysteine is a frequently observed finding in the blood of these patients. Although they may lack some of the vitamins involved in the metabolism of homocysteine, the increased levels of total homocysteine are mainly due to impaired removal of homocysteine from the blood by the kidneys.[14, 15]

It has been suggested that elevated homocysteine is a modifiable, independent risk factor for CAD, stroke and deep vein thrombosis.[16] Studies have also identified elevated homocysteine as a strong independent risk factor for developing various forms of dementia, including Alzheimer's Disease. In one study consisting of 1092 subjects from the Framingham Study, plasma homocysteine levels > 14 µmol/L doubled the risk of Alzheimer's Disease.[17]

A study has indicated plasma tHCY levels are lower in pregnant women than non-pregnant women (mean tHCY is approximately 5-6 µmol/L, values > 10 µmol/L are rarely observed). Increased tHCY is associated with increased risk of pregnancy complications (preeclampsia, recurrent early pregnancy loss, premature delivery, low birth weight, and placental abruption or infarction). Maternal hyperhomocysteinemia is related to birth defects such as neural tube defects, orofacial clefts, club foot and Down's Syndrome.[18]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT Homocysteine assay is a one-step immunoassay for the quantitative determination of total L-homocysteine in human serum or plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

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 iSystem optics.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Kit Contents

ARCHITECT Homocysteine 1L71

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT iSystems. Please contact your local distributor.

REF	1L71-27
Σ	100
MICROPARTICLES	1 x 6.5 mL
CONJUGATE	1 x 8.8 mL
ENZYME	1 x 8.6 mL
REDUCTANT	1 x 21.5 mL

MICROPARTICLES 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 S-adenosyl-L-cysteine (SAC) acridinium-labeled conjugate in citrate buffer with surfactants and protein (bovine) stabilizer. Minimum concentration: 1 ng/mL. Preservative: ProClin 300.

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

REDUCTANT Dithiothreitol (DTT) in citrate buffer.

Other Reagents

MULTI-ASSAY MANUAL DILUENT 1 x 100 mL ARCHITECT Multi-Assay Manual Diluent, REF 7D82-50, containing phosphate buffered saline solution. Preservative: antimicrobial agent.

PRE-TRIGGER SOLUTION ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

WASH BUFFER ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[19-22]

The following warnings and precautions apply to: CONJUGATE	
	
WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: MICROPARTICLES / ENZYME	
Contain sodium azide.	
EUH032	Contact with acids liberates very toxic gas.
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
  - To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.

For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Reagent Storage

When stored and handled as directed, reagents are stable until the expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8°C	Until expiration date	May be used immediately after removal from 2-8°C storage. Store in upright position.
Onboard	System temperature	30 days	Discard after 30 days. Recalibration may be required to obtain maximum onboard reagent stability. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

* Reagents may be stored on or off the ARCHITECT iSystem. If reagents are removed from the system, store them at 2-8˚C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The following ARCHITECT Homocysteine assay files must be installed on the ARCHITECT iSystem prior to performing undiluted or diluted assays:

- tHCY (undiluted protocol)
- tHCY Dil (diluted protocol)

For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.

For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.

For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

Alternate Result Units

Edit assay parameter "Result concentration units" to select an alternate unit.

Conversion formula(s):

(Concentration in µmol/L) x (0.1352) = (Concentration in µg/mL)

Default result unit	Conversion factor	Alternate result unit
µmol/L	0.1352	µg/mL

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay:

Specimen Types	Collection Tubes
Human serum	Serum
	Serum separator tubes
Human plasma	Lithium heparin
	Potassium EDTA

- Other specimen collection tube types have not been tested with this assay.
- Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient specimens.
- The instrument does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - grossly hemolyzed
  - obvious microbial contamination
- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum or plasma.
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- **To minimize increases in homocysteine concentration from synthesis by red blood cells, place all specimens (serum and plasma) on ice after collection and prior to processing.** Serum may clot more slowly and the volume of serum may be reduced as a result of being on ice.[23]

  NOTE: Specimens not placed on ice immediately may exhibit a 10-20% increase in concentration.[24]
- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, centrifuge specimens before testing if
  - they contain fibrin, red blood cells, or other particulate matter,
  - they require repeat testing, or
  - they were frozen and thawed.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Specimen Storage

All specimens must be placed on ice immediately after collection.

Specimen Type	Storage Temperature	Maximum Storage Time
Serum or plasma	On ice	≤ 6 hours
	2-8˚C	≤ 14 days
	≤ - 20˚C or colder	≤ 1 year

If testing will be delayed more than six hours,[23] remove serum or plasma from the clot, red blood cells, or separator gel.

Serum or plasma specimens stored frozen for one year showed no performance difference.[18]

Avoid more than 3 freeze/thaw cycles.

Specimen Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

1L71 ARCHITECT Homocysteine Reagent Kit

Materials Required but not Provided

- ARCHITECT Homocysteine Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 1L71-01 ARCHITECT Homocysteine Calibrators
- 1L71-10 ARCHITECT Homocysteine Controls
- 7D82-50 ARCHITECT Multi-Assay Manual Diluent

- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
- ARCHITECT Replacement Caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- Minimum sample cup volume is calculated by the system and printed on the Orderlist report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

  Maximum number of replicates sampled from the same sample cup: 10
- For the neat (undiluted) protocol:
  - Priority:

    Sample volume for first test: 78 µL

    Sample volume for each additional test from same sample cup: 28 µL
  - ≤ 3 hours on board:

    Sample volume for first test: 150 µL

    Sample volume for each additional test from same sample cup: 28 µL
- For the automated dilution protocol (1:10):
  - Priority:

    Sample volume for first test: 80 µL

    Sample volume for each additional test from same sample cup: 30 µL
  - ≤ 3 hours on board:

    Sample volume for first test: 150 µL

    Sample volume for each additional test from same sample cup: 30 µL
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare ARCHITECT Homocysteine Calibrators and Controls.
  - ARCHITECT Homocysteine Calibrators and Controls should be prepared according to their respective package inserts.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.

    for each calibrator: 5 drops

    for each control: 5 drops
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures

Specimens with a homocysteine value exceeding 50.00 µmol/L are flagged with the code “> 50.00” and may be diluted with the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a 1:10 dilution of the specimen when ordered through the separate assay file "tHCY Dil". This assay file will automatically calculate the concentrations of the specimen before the dilution and report the result.

Manual Dilution Procedure

The testing of a manually diluted sample must be performed using the "tHCY" assay file.

Suggested dilution: 1:10

1. Add 20 µL of the patient specimen to 180 µL of the ARCHITECT Multi-Assay Manual Diluent (7D82-50).
2. The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result.

For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- Test calibrators A-F in replicates of two. The calibrators should be priority loaded.

  A single sample of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the control package insert.
- Calibration Range: 0.0 - 50.0 µmol/L.
- Once an ARCHITECT Homocysteine calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used or
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

Quality Control Procedures

The recommended control requirement for the ARCHITECT Homocysteine assay is that a single sample of each control level be tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures.

The ARCHITECT Homocysteine Control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B.

The ARCHITECT Homocysteine assay belongs to method group 1.

RESULTS

Calculation

The ARCHITECT Homocysteine assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

Measurement Range (Reportable Range)

The measurement range of the ARCHITECT Homocysteine assay is 1.00 µmol/L to 50.00 µmol/L.

LIMITATIONS OF THE PROCEDURE

- If the ARCHITECT Homocysteine assay results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- The following drugs may elevate levels of homocysteine: methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants and 6-azauridine triacetate. The mechanism of action of these drugs affects different parts of the metabolic pathway of homocysteine.[23, 25]
- S-adenosyl-methionine is an antidepressant whose molecular form is similar to S-adenosyl-homocysteine. This drug may interfere with the ARCHITECT Homocysteine assay.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT Homocysteine that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.[26, 27]
- Heterophilic antibodies in human specimens can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[28] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this package insert for specimen limitations.

EXPECTED VALUES

Human EDTA plasma specimens from 300 apparently healthy individuals were evaluated using the ARCHITECT Homocysteine assay. The range of expected values is defined by the central 95% of the observations. The distribution is represented in the following table.*

		Median	Percentile	
Sex	**n**	**(µmol/L)**	**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**

* Representative data; results in individual laboratories may vary from these data. It is recommended that each laboratory establish its own expected range, which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT Homocysteine assay is designed to have an assay precision of ≤ 10% total CV.

A study was performed with guidance from the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP5-A2.[29] Three ARCHITECT Homocysteine Controls and five human plasma panels were assayed using two lots of reagents in replicates of two at two separate times per day for 20 days on two instruments. A new calibration curve was generated for each reagent lot on each day of testing. Data from this study are summarized in the following table.*

		Reagent		Mean	Within Run		Total	
Sample	**Instrument**	**Lot**	**n**	**(µmol/L)**	**SD**	**%CV**	**SD**	**%CV**
Low Control	1	1	80	7.40	0.26	3.5	0.43	5.9
	2	2	80	7.58	0.18	2.4	0.25	3.3
Medium Control	1	1	80	13.21	0.26	2.0	0.64	4.8
	2	2	80	13.37	0.24	1.8	0.41	3.0
High Control	1	1	80	26.77	0.63	2.3	1.09	4.1
	2	2	80	25.73	0.47	1.8	0.73	2.9
Panel 1	1	1	80	4.78	0.19	4.0	0.30	6.3
	2	2	80	4.71	0.13	2.8	0.18	3.8
Panel 2	1	1	80	11.03	0.22	2.0	0.48	4.3
	2	2	80	10.89	0.13	1.2	0.23	2.1
Panel 3	1	1	80	17.60	0.43	2.4	0.77	4.4
	2	2	80	17.29	0.27	1.6	0.46	2.6
Panel 4	1	1	80	35.40	0.79	2.2	1.19	3.4
	2	2	80	34.83	0.71	2.0	0.87	2.5
Panel 5	1	1	80	41.84	0.71	1.7	1.35	3.2
	2	2	80	41.46	0.68	1.6	1.12	2.7

* Representative data; results in individual laboratories may vary from these data.

Dilution

The ARCHITECT Homocysteine assay is designed to have a mean recovery of 100 ± 15% of the expected result for diluted specimens.

A dilution study was performed by diluting high concentration homocysteine EDTA plasma specimens with ARCHITECT Wash Buffer. The concentration of homocysteine was determined for each dilution of sample using the ARCHITECT Homocysteine assay, and the resulting percent recovery was calculated. A subset of the dilutions that were performed on each sample from this study are summarized in the following table. Mean recovery across the entire set of dilutions for each sample equals 103.9%, 106.2%, and 104.1%, respectively, for samples 1, 2, and 3 shown below.*

Sample ID	**Dilution Factor**	**Expected Value (µmol/L)**	**Mean Observed Value (µmol/L)**	**% Recovery[a]**
1	Undiluted	41.97	41.97	--
	1:1.25	33.58	34.11	101.6
	1:2	20.99	21.77	103.7
	1:5	8.39	9.07	108.1
	1:10	4.20	4.62	110.0
2	Undiluted	44.23	44.23	--
	1:1.25	35.38	36.31	102.6
	1:2	22.11	22.81	103.1
	1:5	8.85	9.98	112.8
	1:10	4.42	5.15	116.5
3	Undiluted	45.79	45.79	--
	1:1.25	36.63	36.55	99.8
	1:2	22.90	23.56	102.9
	1:5	9.16	10.35	113.1
	1:10	4.58	4.94	107.8

[a] $\% \text{ Recovery} = \frac{\text{Mean Observed Value (µmol/L)}}{\text{Mean Expected Value (µmol/L)}} \times 100$

* Representative data; results in individual laboratories may vary from these data.

Autodilution Verification

Recovery performance was evaluated for the 1:10 autodilution method of the ARCHITECT Homocysteine assay versus the 1:10 manual dilution method using three human EDTA plasma specimens with homocysteine levels that were greater than the ARCHITECT Homocysteine Calibrator F (50.0 µmol/L). Two replicates each of the autodiluted and manually diluted sample were assayed on one instrument using the ARCHITECT Homocysteine assay. The percent recovery results are summarized in the following table.*

Sample ID	Mean Automated Diluted Value (µmol/L)	Mean Manually Diluted Value (µmol/L)	% Recovery[a]
1	40.19	44.06	91.2
2	41.10	44.85	91.6
3	40.56	44.46	91.2

$$[a]\ \%\ \text{Recovery} = \frac{\text{Mean Automated Diluted Value (µmol/L)}}{\text{Mean Manually Diluted Value (µmol/L)}} \times 100$$

* Representative data; results in individual laboratories may vary from these data.

Sensitivity

Sensitivity is defined as the limit of detection (LoD). The ARCHITECT Homocysteine assay is designed to have a limit of detection of ≤ 1.0 µmol/L. The limit of blank (LoB) and LoD of the ARCHITECT Homocysteine assay were determined based on guidance from the CLSI Protocol EP17-A[30] using proportions of false positives (α) less than 5% and false negatives (β) less than 5%. These determinations were performed using one blank (120 replicates) and five low level homocysteine samples (40 replicates each); LoB = 0.30 µmol/L and LoD = 0.64 µmol/L.*

* Representative data; results in individual laboratories may vary from these data.

Specificity

The specificity of the ARCHITECT Homocysteine assay was determined by studying the cross-reactivity of compounds whose chemical structure or concurrent usage may potentially interfere with the ARCHITECT Homocysteine assay. A study based on guidance from CLSI Protocol EP7-A2[31] was performed for the ARCHITECT Homocysteine assay. Specificity of the assay was determined by spiking solutions of each of the following compounds into human EDTA plasma specimens with homocysteine values ranging from 4.83 µmol/L to 43.70 µmol/L. Mean percent cross-reactivity at the levels indicated for each compound are summarized in the following table.*

Test Compound	Concentration (mM)	Mean % Cross- Reactivity[a]
S-Adenosyl-L-Methionine	0.5	11.78[b]
L-Cysteine	100	0.01
L-Cystathionine	0.5	0.30
Adenosine	5.0	0.72
Glutathione	100	0.003
DL-Homocysteine Thiolactone	0.25	3.22

$$[a]\ \%\ \text{Cross- Reactivity} = \frac{\text{Observed Test Concentration (µmol/L) - Control Concentration (µmol/L)}}{\text{Concentration of Cross-Reactant (µmol/L)}} \times 100$$

[b] Refer to the **LIMITATIONS OF THE PROCEDURE** section of this package insert.

* Representative data; results in individual laboratories may vary from these data.

Interference

Potential interference in the ARCHITECT Homocysteine assay from the following compounds is designed to have a mean recovery of 100 ± 10% of the expected homocysteine concentration at the levels indicated. Studies based on guidance from the CLSI Protocol EP7-A2[31] were performed for the ARCHITECT Homocysteine assay. EDTA plasma specimens with homocysteine levels across the assay range of 1.00 to 50.00 µmol/L were supplemented with the following potentially interfering compounds. The mean recovery observed in EDTA plasma specimens during these studies ranged from 94.4% to 104.5%.*

Potentially Interfering Substance	Concentration
Bilirubin	20 mg/dL
Hemoglobin	1000 mg/dL
Low Protein	3 g/dL
High Protein	12 g/dL
Triglycerides	6000 mg/dL
Heparin	1 U/mL

* Representative data; results in individual laboratories may vary from these data.

Method Comparison

The ARCHITECT Homocysteine assay is designed to have a slope of 1.0 ± 0.1 and a correlation coefficient (r) of ≥ 0.90 for plasma samples when compared to AxSYM Homocysteine.

A correlation study based on guidance from CLSI Protocol EP9-A2[32] was performed on 456 EDTA plasma specimens to compare the ARCHITECT Homocysteine assay to the AxSYM Homocysteine assay. Data from this study were analyzed using the Passing-Bablok[a] regression method and are summarized in the following table and graph.*

ARCHITECT Homocysteine vs. AxSYM Homocysteine

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

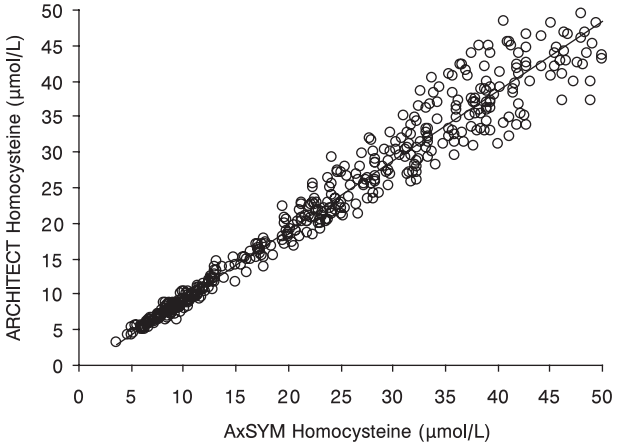
Specimen Range (ARCHITECT): 3.18 µmol/L to 49.39 µmol/L

Specimen Range (AxSYM): 3.70 µmol/L to 49.94 µmol/L

[a] A linear regression method with no special assumptions regarding the distribution of the samples and measurement errors.[33]

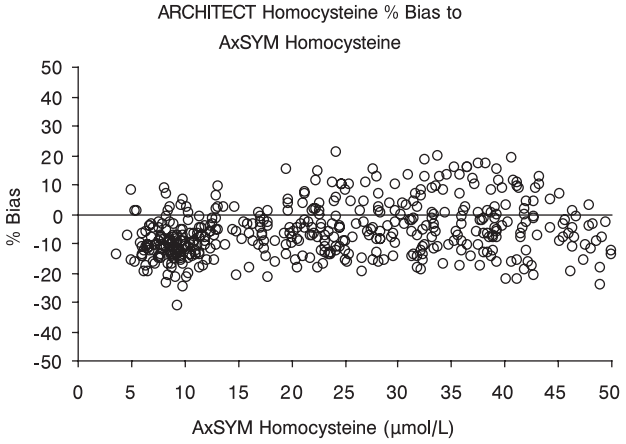
ARCHITECT Homocysteine vs.

AxSYM Homocysteine



* Representative data; variables such as differences in sampling size and sample population may impact the correlation of the assay, therefore, results in individual laboratories may vary from these data.

A bias analysis of ARCHITECT Homocysteine vs. AxSYM Homocysteine was performed on the same 456 Method Comparison specimens in the range of 3.70 to 49.94 µmol/L. The following representative data are provided to aid in understanding the difference between the two assays. The average percent bias exhibited by ARCHITECT vs. AxSYM in this study was -5.94%. The 95% confidence interval of that average percent bias is -6.77% to -5.12%. Results of the study are summarized in the following graph.*



* Representative data; results in individual laboratories may vary from these data.

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Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number
DISTRIBUTED IN THE USA BY	Distributed in the USA by
ENZYME	Enzyme
INFORMATION FOR USA ONLY	Information needed for United States of America only
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
MULTI-ASSAY MANUAL DILUENT	Multi-Assay Manual Diluent
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCED FOR ABBOTT BY	Produced for Abbott by
PRODUCT OF UK	Product of United Kingdom
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REDUCTANT	Reductant
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
TRIGGER SOLUTION	Trigger Solution
WARNING: SENSITIZER	Warning: May cause an allergic reaction.
WASH BUFFER	Wash Buffer

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