Read Highlighted Changes: Revised August 2018.

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.

NAME

ARCHITECT BNP

INTENDED USE

The ARCHITECT BNP assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of human B-type natriuretic peptide (BNP) in human EDTA plasma on the ARCHITECT iSystem. BNP values are used as an aid in the diagnosis and assessment of severity of heart failure.

SUMMARY AND EXPLANATION OF THE TEST

Heart failure is a syndrome caused by a variety of conditions such as coronary artery disease, hypertension, valve disease, myocarditis and others. Common symptoms of heart failure include shortness of breath, coughing under exertion, swelling of appendages, and dizziness. Heart failure is better defined as the progressive inability of the heart ventricles to pump blood out to the lungs and/or the extremities. Heart failure is either systolic or diastolic or a combination of both. Severity is usually classified into four classes defined by the New York Heart Association (NYHA class I-IV).

BNP is one member of the family of natriuretic peptides that were initially discovered by de Bold, et al.[1] Although BNP was first isolated from porcine brain tissue (originally named brain natriuretic peptide),[2] the heart has been determined to be the major source.[3] BNP is synthesized and released into the blood in response to volume overload or conditions that cause ventricular stretch, to control fluid and electrolyte homeostasis by interaction with the renin-angiotensin-aldosterone system (RAAS).[3, 4] PreproBNP (134 amino acids) is synthesized in the cardiac myocytes and is processed to a proBNP (108 amino acids) precursor molecule. The proBNP is subsequently cleaved into physiologically active BNP (32 amino acids), and a degradation fragment NT-proBNP (76 amino acids).[5, 6] BNP, NT-proBNP, and a higher molecular weight form have been detected in peripheral blood.[7, 8] BNP is cleared from the circulation, with a half-life (t½) of approximately 23 minutes, by specific cellular receptors and neutral endopeptidases.[9] Numerous studies have indicated that BNP can be used for patient diagnosis, prognosis and therapy monitoring. Levels of BNP have been shown to be elevated in patients with cardiac dysfunction.[10, 11] Plasma BNP levels provide clinically useful information concerning the diagnosis and management of left ventricular dysfunction and heart failure, which complements other diagnostic testing procedures (e.g., electrocardiograms, chest x-rays, and echocardiograms).[12, 13] BNP levels can be used to assess the severity of heart failure, as demonstrated by the correlation with New York Heart Association classifications.[14] Plasma BNP levels also increase with decreasing physiological functional capacities, as measured by left ventricular ejection fraction (LVEF) or exercise-based evaluations.[15, 16] The European Society of Cardiology has included the use of natriuretic peptides (e.g., BNP) testing in their guidelines for the diagnosis or rule out of heart failure.[17]

Others have suggested that BNP has utility in the stratification of patients with heart failure and acute coronary syndrome (ACS). Elevated levels of BNP in heart failure patients predict disease progression and increased morbidity and mortality.[18-20] Studies also suggest ACS patients with increased BNP levels have a higher rate of cardiac complications and higher mortality post myocardial infarction.[21, 22] Preliminary studies have reported the use of BNP measurements to optimize patient treatment / management for heart failure.[23-25] Nesiritide (Natrecor), recombinant BNP has been used for treatment in patients with acute, decompensated heart failure.[26] The efficacy of BNP monitoring, pre- and post-treatment with Natrecor, has been studied.[27] Measurements of BNP two hours or more post-treatment detect only the endogenous levels of BNP.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT BNP assay is a two-step immunoassay for the quantitative determination of human B-type natriuretic peptide (BNP) in human EDTA plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample, and anti-BNP coated paramagnetic microparticles are combined. The BNP present in the sample binds to the anti-BNP coated microparticles.
2. After washing, anti-BNP acridinium-labeled conjugate is added to create a reaction mixture.
3. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of BNP 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 BNP 8K28

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

REF	8K28-28	8K28-36
Σ	100	500
MICROPARTICLES	1 x 6.6 mL	1 x 26.8 mL
CONJUGATE	1 x 5.9 mL	1 x 26.0 mL
SPECIMEN DILUENT	1 x 8.9 mL	1 x 45.4 mL

MICROPARTICLES Anti-BNP (mouse, monoclonal) coated microparticles in TRIS buffer with protein (bovine, mouse) stabilizers. Minimum concentration: 0.07% solids. Preservatives: sodium azide and ProClin 950.

CONJUGATE Anti-BNP (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizer. Minimum concentration: 0.1 µg/mL. Preservatives: sodium azide and ProClin 300.

SPECIMEN DILUENT Specimen Diluent containing TRIS buffer with protein (bovine) stabilizer. Preservatives: sodium azide and ProClin 950.

Other Reagents

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.[28-31]

The following warnings and precautions apply to: **MICROPARTICLES** / **SPECIMEN DILUENT**

WARNING	Contains methylisothiazolones and sodium azide.
H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
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: **CONJUGATE**

WARNING	Contains methylisothiazolones, polyethylene glycol octylphenyl ether and sodium azide.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice / attention.
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.

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.
On board	System temperature	30 days	After 30 days, the reagent kit must be discarded. 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 any reagent 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 ARCHITECT BNP assay file must be installed on the ARCHITECT iSystem prior to performing the assay. The BNP STAT assay file must be installed on an ARCHITECT iSystem with STAT protocol capability prior to performing the assay.

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

The default result unit for the ARCHITECT BNP assay is pg/mL. The corresponding SI result unit is pmol/L. The conversion factor used by the system is 0.2887.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay:

	Collection Tubes
Specimen Types	**Plastic**
Plasma	EDTA

- Other sample types, including serum, citrate plasma and heparin plasma are not recommended and have not been evaluated.
- Samples should be collected in **plastic collection tubes**, because the BNP molecule has been shown to be unstable in glass containers.[32, 33]

  Follow the manufacturer's processing instructions for plasma collection tubes.
- 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:
  - grossly hemolyzed
  - collected in glass containers
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- 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.[34]
- To ensure consistency in results, centrifuge specimens before testing if
  - they contain fibrin, red blood cells, or other particulate matter 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

Specimen Type	Storage Temperature	Maximum Storage Time
Plasma	Room temperature	Test within 4 hours of collection
	2-8°C	Test within 24 hours of collection
	-20°C or colder	≤ 3 months
Whole blood	Room temperature	Test within 4 hours of collection
	2-8°C	Test within 24 hours of collection

- Whole blood samples, stored at 2-8°C must be tested within 24 hours of collection.
- Whole blood samples, stored at room temperature must be tested within 4 hours of collection.
- Plasma samples, stored at 2-8°C must be tested within 24 hours of collection.
- Plasma samples, stored at room temperature must be tested within 4 hours of collection.
- If samples cannot be tested within the given times for room temperature or 2-8°C storage, they may be separated by centrifugation and frozen for up to 3 months at -20°C or colder in plastic tubes.
- Avoid multiple freeze/thaw cycles.
- Samples may undergo up to 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

8K28 ARCHITECT BNP Reagent Kit

Materials Required but not Provided

- ARCHITECT BNP Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 8K28 ARCHITECT BNP Calibrators
- 8K28 ARCHITECT BNP Controls
- 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 the 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 or the ARCHITECT iSystem with STAT protocol capability.
  - 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 that adequate sample cup volume is present prior to running the test.
- Maximum number of replicates sampled from the same sample cup: 10
  - Priority:

    Sample volume for the first test: 150 µL

    Sample volume for each additional test from same sample cup: 100 µL
- ≤ 3 hours on board:

  Sample volume for the first test: 150 µL

  Sample volume for each additional test from same sample cup: 100 µL
- To minimize the effects of evaporation all samples (patient specimens, calibrators and controls) must be tested within 3 hours of being placed on board the ARCHITECT iSystem.
  - If using primary or aliquot tubes, use the sample gauge to ensure that sufficient patient specimen is present.
- Prepare ARCHITECT BNP Calibrators and Controls.
  - Mix Calibrators and Controls by gentle inversion before use.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
- Recommended volumes:

  for each calibrator: Minimum 5 drops

  for each control: Minimum 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 BNP value exceeding 5000.0 pg/mL are flagged with the code ">5000.0 pg/mL" and may be diluted using the Automated Dilution Protocol.

Automated Dilution Protocol

The system performs a 1:5 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.

Retest of samples must be performed within assay specimen handling limits to ensure optimal BNP recovery (refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert).

Manual Dilution Procedure

The Manual Dilution Feature of the Assay Parameter is set to ON primarily for use when performing dilution linearity studies.

NOTE: The Automated Dilution Protocol is preferred when diluting specimens due to the known instability of the BNP analyte. If performing manual dilutions, the specimen handling guidelines must be followed (refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert).

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

Calibration

- Test Calibrators A-F in duplicate. 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 respective control package insert.
- Calibration Range: 0 – 5000 pg/mL.
- Once an ARCHITECT BNP 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 BNP 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.
- Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated sample results are invalid and the samples 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 BNP assay belongs to method group 1.

RESULTS

Calculation

The ARCHITECT BNP assay utilizes a point-to-point data reduction method 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 for the ARCHITECT BNP assay is 10 to 5000 pg/mL.

LIMITATIONS OF THE PROCEDURE

- EDTA plasma, collected in plastic tubes, should be used for this assay. The use of glass collection tubes, or other sample types, such as serum or plasma with other anticoagulants, is not recommended.
- For diagnostic purposes, the ARCHITECT BNP results should be used in conjunction with other clinical data; e.g., symptoms, medical history, etc. If BNP results are not consistent with other clinical observations, additional information may be required for diagnosis.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[35] Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.[36] ARCHITECT BNP reagents contain a component that reduces the effect of HAMA reactive specimens. Additional clinical or diagnosis information may be required to determine patient status.

- Heterophilic antibodies in human plasma can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. The presence of heterophilic antibodies in a patient may cause anomalous values to be observed.[37, 38]
- ARCHITECT BNP results should not be used interchangeably with other manufacturers' methods for BNP or NT-proBNP determinations.
- Measurements of BNP should occur prior to Nesiritide (Natrecor), recombinant BNP treatment and 2 hours post-treatment.[27]
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this package insert for additional information.

EXPECTED VALUES

Non-Heart Failure Population

Plasma samples from 890 individuals (465 females, 425 males) who had not been diagnosed with heart failure were tested with the AxSYM BNP assay. This population included non-hospitalized patients with renal disease (not on dialysis), diabetes, hypertension and chronic obstructive pulmonary disease. BNP levels for the patients with renal disease, diabetes, hypertension and chronic obstructive pulmonary disease were not statistically different from the population of apparently healthy individuals. The data from this study are summarized in the following table.*

Non-Heart Failure Population - All (Age Group)

	All	< 45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sample Size (N=)	890	205	146	171	248	120
Median (pg/mL)	21	17	9	24	23	31
Mean (pg/mL)	39	28	21	37	47	63
SD (pg/mL)	66	36	30	48	80	109
95th Percentile	135	85	87	119	160	254
Percentage < 100 pg/mL	91.5%	96.6%	95.2%	94.2%	87.1%	83.3%
Minimum (pg/mL)	0	0	0	0	0	0
Maximum (pg/mL)	907	263	142	380	907	837

Non-Heart Failure Population - Males (Age Group)

	All	< 45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sample Size (N=)	425	107	71	94	115	38
Median (pg/mL)	14	12	1	17	21	37
Mean (pg/mL)	30	23	9	26	47	49
SD (pg/mL)	61	34	14	45	96	51
95th Percentile	104	73	40	80	150	121
Percentage < 100 pg/mL	94.8%	97.2%	100.0%	97.9%	88.7%	89.5%
Minimum (pg/mL)	0	0	0	0	0	0
Maximum (pg/mL)	907	200	57	380	907	254

Non-Heart Failure Population - Females (Age Group)

	All	< 45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sample Size (N=)	465	98	75	77	133	82
Median (pg/mL)	26	23	23	37	23	25
Mean (pg/mL)	46	34	34	51	46	69
SD (pg/mL)	70	37	36	48	63	126
95th Percentile	150	89	111	155	159	266
Percentage < 100 pg/mL	88.4%	95.9%	90.7%	89.6%	85.7%	80.5%
Minimum (pg/mL)	0	0	0	0	0	0
Maximum (pg/mL)	837	263	142	230	374	837

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

Due to demographic population differences, the reference range should be established at each laboratory.

Heart Failure Population

Plasma samples from 693 patients with diagnosed heart failure (231 females, 462 males) were tested with the AxSYM BNP assay. All patients in this population were categorized according to the functional classification system published by the New York Heart Association (NYHA).[39] This system divides heart failure patients into one of four categories of increasing disease progression (classes I to IV) based upon a subjective assessment of the patient's clinical signs and symptoms. The data from this study are summarized in the following table.*

Heart Failure Population - All

		NYHA Functional Class			
	All	I	II	III	IV
Sample Size (N=)	693	124	319	190	60
Median (pg/mL)	298	133	266	335	1531
Mean (pg/mL)	578	320	432	656	1635
SD (pg/mL)	771	388	574	841	1097
5th Percentile	14	9	15	12	188
95th Percentile	2154	1257	1534	2516	>4000
Percentage ≥ 100 pg/mL	74.2%	58.1%	73.0%	79.0%	98.3%
Minimum (pg/mL)	0	3	0	0	14
Maximum (pg/mL)	>4000	1651	>4000	>4000	>4000

Heart Failure Population - Males

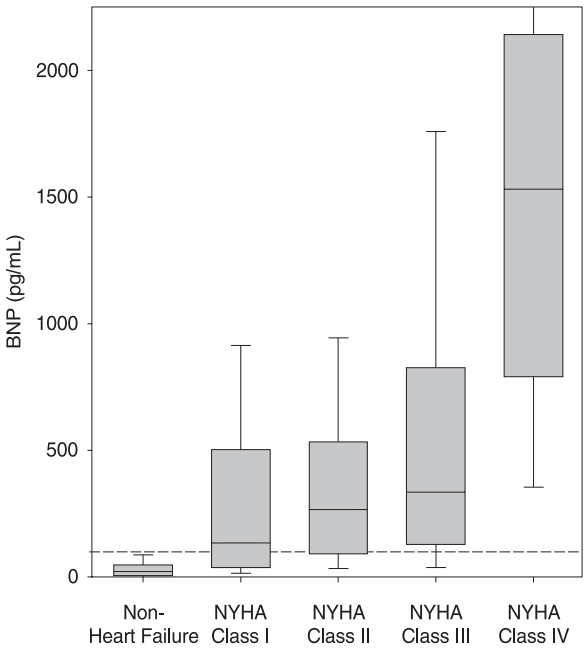
		NYHA Functional Class			
	All	I	II	III	IV
Sample Size (N=)	462	94	215	121	32
Median (pg/mL)	268	122	258	293	1645
Mean (pg/mL)	524	314	409	597	1646
SD (pg/mL)	719	390	539	821	1032
5th Percentile	12	9	14	22	265
95th Percentile	1976	1281	1356	2288	3654
Percentage ≥ 100 pg/mL	71.0%	56.4%	70.7%	76.0%	96.9%
Minimum (pg/mL)	0	3	0	0	14
Maximum (pg/mL)	>4000	1408	3782	>4000	>4000

Heart Failure Population - Females

		NYHA Functional Class			
	All	I	II	III	IV
Sample Size (N=)	231	30	104	69	28
Median (pg/mL)	385	174	298	466	1408
Mean (pg/mL)	685	341	481	760	1623
SD (pg/mL)	858	388	641	870	1186
5th Percentile	16	14	21	12	244
95th Percentile	2593	1022	2031	2718	>4000
Percentage ≥ 100 pg/mL	80.5%	63.3%	77.9%	84.1%	100.0%
Minimum (pg/mL)	0	10	0	0	173
Maximum (pg/mL)	>4000	1651	>4000	>4000	>4000

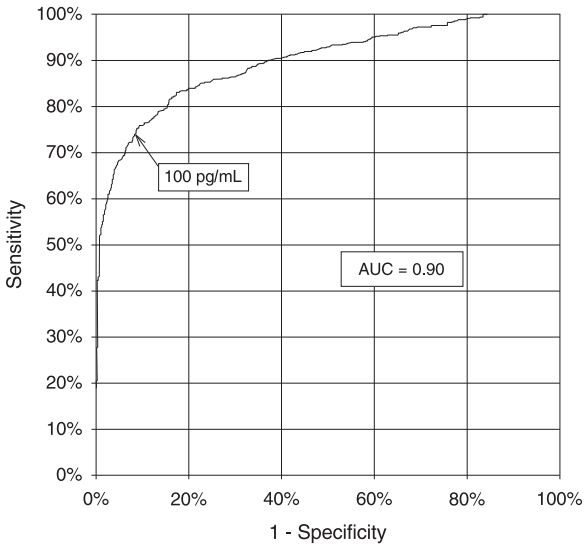
* Representative data; results in individual laboratories may vary from these data.

A box and whiskers plot of the clinical study population, broken down by NYHA classification, is presented in the following graph. The dashed line represents 100 pg/mL, the suggested decision threshold for the AxSYM BNP assay. In support of previous literature reports,[14] these data show a progressive increase in BNP concentrations with increases in NYHA classifications. This analysis indicates that BNP measurements provide objective information for use in the assessment of the severity of heart failure.



Data from the above clinical study were used to generate the Receiver Operating Characteristic (ROC) curve of BNP decision thresholds versus clinical sensitivity and clinical specificity as shown in the following graph. At a decision threshold of 100 pg/mL, the BNP assay demonstrated a clinical sensitivity and specificity of 74.2% and 91.5%, respectively, in this study. The area under the curve (AUC) is 0.90 (0.86 to 0.92, 95% CI).

BNP ROC Curve
Heart Failure Population (n=693) and
Non-Heart Failure Population (n=890)



The ARCHITECT BNP Calibrators are traceable to an internal reference standard that has been prepared gravimetrically with synthetic BNP. The internal reference standard correlates to the AxSYM BNP assay with a decision threshold of 100 pg/mL. There is no internationally recognized BNP standard available at this time.

An age-matched analysis of the heart failure and non-heart failure populations was performed based on the data published by the American Heart Association in the 2000 Heart and Stroke Statistical Update[40] and according to the age structure of the United States population.[41] The age distributions in the intended use population are approximately as follows: individuals less than 45 years old comprise 9%, individuals 45-54 years old comprise 11%, individuals 55-64 years old comprise 22%, individuals 65-74 years old comprise 26%, and individuals 75 years and older comprise 32%. The resulting combined AUC is 0.87 (0.85 to 0.90, 95% CI).

The clinical sensitivity and specificity using a decision threshold of 100 pg/mL is presented in the following table.*

	Males (Age Group)					
	All	< 45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sensitivity	71.0%	47.1%	57.1%	57.3%	70.6%	86.1%
	(328/462)	(8/17)	(24/42)	(51/89)	(115/163)	(130/151)
95% Confidence Interval	66.6 to 75.1%	23.0 to 72.2%	41.0 to 72.3%	46.4 to 67.7%	62.9 to 77.4%	79.5 to 91.2%
Specificity	94.8%	97.2%	100.0%	97.9%	88.7%	89.5%
	(403/425)	(104/107)	(71/71)	(92/94)	(102/115)	(34/38)
95% Confidence Interval	92.3 to 96.7%	92.0 to 99.4%	94.9 to 100.0%	92.5 to 99.7%	81.5 to 93.8%	75.2 to 97.1%

	Females (Age Group)					
	All	< 45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sensitivity	80.5%	44.4%	73.3%	50.0%	80.6%	91.7%
	(186/231)	(4/9)	(11/15)	(13/26)	(58/72)	(100/109)
95% Confidence Interval	74.8 to 85.4%	13.7 to 78.8%	44.9 to 92.2%	29.9 to 70.1%	69.5 to 88.9%	84.9 to 96.2%
Specificity	88.4%	95.9%	90.7%	89.6%	85.7%	80.5%
	(411/465)	(94/98)	(68/75)	(69/77)	(114/133)	(66/82)
95% Confidence Interval	85.1 to 91.2%	89.9 to 98.9%	81.7 to 96.2%	80.6 to 95.4%	78.6 to 91.2%	70.3 to 88.4%

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

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT BNP assay is designed to have an upper 95% Confidence Interval (CI) imprecision of ≤ 12% total CV.

A study was performed with the ARCHITECT BNP assay based on guidance from the National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP5-A2.[42] A three-member panel and the ARCHITECT BNP Controls were assayed in replicates of two at two separate times of the day for 20 days. Testing was performed on two ARCHITECT Systems using a single calibration on each instrument. Data from this study are summarized in the following table.*

Sample	Reagent Lot	Instrument	n	Mean Conc. (pg/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
Panel 1	1	1	80	183.6	6.9	3.8	9.8	5.3
	2	2	80	180.1	10.0	5.6	12.1	6.7
Panel 2	1	1	80	1004.4	26.4	2.6	41.1	4.1
	2	2	80	961.6	44.8	4.7	53.7	5.6
Panel 3	1	1	80	3812.1	91.3	2.4	153.5	4.0
	2	2	80	3645.1	138.4	3.8	188.9	5.2
Low Control	1	1	80	92.2	3.2	3.5	4.0	4.4
	2	2	80	87.8	3.6	4.0	4.1	4.7
Medium Control	1	1	80	504.3	9.3	1.8	13.5	2.7
	2	2	80	494.1	13.1	2.6	17.2	3.5
High Control	1	1	80	3572.1	32.6	0.9	62.0	1.7
	2	2	80	3423.0	80.1	2.3	112.3	3.3

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

Dilution Linearity

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

A dilution linearity study was performed by diluting high concentration BNP specimens with the ARCHITECT BNP Specimen Diluent. The concentration of BNP was determined for each dilution of sample and the percent (%) recovery was calculated. Data from this study are summarized in the following table.*

Sample	Final Dilution Factor	Value Obtained (pg/mL)	% Recovery[a]
1	Undiluted	4772	-
	1.33	3489	98
	2	2426	102
	5	921	97
2	Undiluted	3791	-
	1.33	2679	94
	2	1918	101
	5	675	89
3	Undiluted	4071	-
	1.33	3107	102
	2	1556	77
	5	739	91
4	Undiluted	4985	-
	1.33	3548	95
	2	2237	90
	5	911	91
5	Undiluted	4011	-
	1.33	3061	102
	2	2032	101
	5	716	89

Mean recovery across the five diluted samples shown above = 95%

$$^{a}\,\%\ \text{Recovery} = \frac{\text{BNP Value Obtained (pg/mL)}}{\text{(Undiluted Value / Dilution Factor)}} \times 100$$

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

Analytical Sensitivity

The ARCHITECT BNP assay is designed to have an analytical sensitivity of ≤ 10 pg/mL.

Analytical sensitivity is defined as the concentration calculated as the mean plus two standard deviations of replicates of the ARCHITECT BNP Calibrator A (0 pg/mL). The analytical sensitivity (low-linearity) is defined in the ARCHITECT BNP assay parameters as 10 pg/mL.

Analytical Specificity

The specificity of the ARCHITECT BNP assay is designed to be ≤ 10 pg/mL of measured BNP when tested with human ANP, Angiotensin I, II and III, CNP, and NT-proBNP at the following concentrations. Each potential cross-reactant was added to protease-inhibitor treated plasma and then assayed. Data from this study are summarized in the following table.*

Potential Cross-reactant	Concentration (pg/mL)	BNP Concentration[a] (pg/mL)
ANP	1000	< 10
Angiotensin I	600	< 10
Angiotensin II	600	< 10
Angiotensin III	1000	< 10
CNP	1000	< 10
NT-proBNP (1-76)	1000	< 10

[a] Cross-reactivity = BNP Value Obtained (pg/mL) - Endogenous BNP Level (pg/mL)

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

Carryover

Carryover from a sample with a high BNP value (approximately 25000 pg/mL) to an adjacent sample of ARCHITECT BNP Calibrator A (0 pg/mL) was determined to be below the analytical sensitivity of the assay.*

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

Interference

Potential interference in the ARCHITECT BNP assay from the following compounds is designed to be ≤ 10%.

Potential interference was evaluated by a study based upon guidance from NCCLS Protocol EP7-A.[43] Specimens were supplemented with various drugs and potentially interfering substances (triglycerides, hemoglobin, bilirubin, and total protein) at the levels indicated in the following tables. The average recovery observed during the study ranged from 92 to 110%.*

Drug	Drug Concentration	Drug	Drug Concentration
Acetaminophen	30 µg/mL	Indomethacin	36 µg/mL
Acetylsalicylic Acid	600 µg/mL	Isosorbide Dinitrate	150 ng/mL
Amiodarone	6 µg/mL	Lisinopril	4 µg/mL
Amlodipine besylate	100 ng/mL	Lovastatin	20 µg/mL
Ampicillin	53 µg/mL	Methyldopa	15 µg/mL
Ascorbic Acid	40 µg/mL	Nicotine	1 µg/mL
Atenolol	10 µg/mL	Nifedipine	400 ng/mL
Caffeine	60 µg/mL	Nitrofurantoin	4 µg/mL
Captopril	5 µg/mL	Nitroglycerine	500 ng/mL
Chloramphenicol	50 µg/mL	Oxazepam	5 µg/mL
Clopidogrel Bisulphate	2.5 µg/mL	Oxytetracycline	15 µg/mL
Cyclosporine	2.5 µg/mL	Phenobarbital	100 µg/mL
Diclofenac	50 µg/mL	Phenytoin	50 µg/mL
Digoxin	2 ng/mL	Probenecid	600 µg/mL
Diltiazem	40 µg/mL	Procainamide	24 µg/mL
Dipyridamole	80 µg/mL	Propranolol	2 µg/mL
Dobutamine	100 µg/mL	Quinidine	12 µg/mL
Dopamine	900 ng/mL	Simvastatin	16 µg/mL
Enalapril Maleate	300 ng/mL	Spironolactone	600 ng/mL
Erythromycin	60 µg/mL	Sulfamethoxazole	400 µg/mL
Fenofibrate	45 µg/mL	Trandolapril	40 µg/mL
Furosemide	60 µg/mL	Trimethoprim	40 µg/mL
Heparin	8 U/mL	Verapamil	2 µg/mL
Hydralazine	6.4 µg/mL	Warfarin	20 µg/mL
Hydrochlorothiazide	6 µg/mL		

Potentially Interfering Substance	Concentration
Triglycerides	3000 mg/dL
Hemoglobin	500 mg/dL
Bilirubin	20 mg/dL
Total Protein	3 g/dL
Total Protein	12 g/dL

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

Method Comparison

The ARCHITECT BNP assay is designed to have a slope of 1.0 ± 0.2 and a correlation coefficient of (r) ≥ 0.90 when compared to AxSYM BNP assay.

A study was performed with guidance from NCCLS Protocol EP9-A2[44] to compare the ARCHITECT BNP assay to the AxSYM BNP assay. EDTA plasma samples from 171 individuals (128 heart failure patients, 43 non-heart failure individuals) were tested with both assays. These samples were collected from populations of individuals with and without heart failure. The results from the Passing-Bablok[45] linear regression analysis are summarized in the following table.*

ARCHITECT BNP vs. AxSYM BNP					
Regression Method	Specimen Type	n	Correlation Coefficient	Intercept (95% CI)	Slope (95% CI)
Passing-Bablok †	EDTA Plasma	171	0.96	-38.32 (-48.26 to -28.50)	1.03 (0.98 to 1.09)

Sample Range (ARCHITECT): 0 – 3702 pg/mL

Sample Range (AxSYM): 50 – 3103 pg/mL

†A linear regression method with no special assumptions regarding the distribution of samples and measurement errors.

* Representative data; variables such as differences in sampling size and population may impact the correlation of the assay, therefore, 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
INFORMATION FOR USA ONLY	Information needed for United States of America only
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCED FOR ABBOTT BY	Produced for Abbott by
PRODUCT OF USA	Product of USA
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
SPECIMEN DILUENT	Specimen Diluent
TRIGGER SOLUTION	Trigger Solution
WARNING: EYE IRRITANT	Warning: Causes serious eye irritation.
WARNING: SENSITIZER	Warning: May cause an allergic reaction.
WASH BUFFER	Wash Buffer

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