Intended Use: The LOCI B-type Natriuretic Peptide method is an *in vitro* diagnostic test for the quantitative measurement of B-type natriuretic peptide in human plasma on the Dimension Vista® System. Measurements of B-type natriuretic peptide are used as an aid in the diagnosis and assessment of the severity of heart failure. In patients with acute coronary syndromes (ACS), this test, in conjunction with other known risk factors, can be used to predict survival and the likelihood of future heart failure.

Summary: Heart failure is an important clinical syndrome, which compromises left ventricular systolic or diastolic function or a combination of both. Heart failure occurs when the heart is unable to pump blood at a rate sufficient for metabolic requirements. Its most common causes are coronary artery disease, hypertension, valvular heart diseases and cardiomyopathies. Accurate and early diagnosis is important since effective therapeutic interventions (e.g. angiotensin converting enzyme inhibitors, beta-blockers) are available, which improve both morbidity and mortality. Based on clinical signs and symptoms, the severity of heart failure is classified into 4 classes of increasing disease progression according to the New York Heart Association classification (NYHA class I – IV).[1, 2]

The natriuretic peptide system is a family of structurally similar but genetically distinct peptides, which include atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) of myocardial cell origin and C-type natriuretic peptide (CNP) of endothelial cell origin. These peptides are characterized by a common 17 amino acid ring structure with a disulfide bond between two cysteine residues.[3-5] The cardiac natriuretic peptides are the naturally occurring antagonists of the rennin-angiotensin-aldosterone system and of the sympathetic nervous system. They promote natriuresis and diuresis, act as vasodilators, and exert antimitogenic effects on cardiovascular tissues.[6] ANP and BNP are secreted by the heart in response to hemodynamic stress. Increased levels of BNP are produced mainly in response to left ventricular wall stretch and volume overload. ANP and BNP are expressed predominantly in the atria and ventricles, respectively, and are important in regulation of blood pressure, electrolyte and volume homeostasis.[6-10]

The cardiac natriuretic peptide system is activated to its highest degree in ventricular dysfunction and has an important role in maintaining the compensated state of asymptomatic heart failure and delaying disease progression. BNP is synthesized within the cardiomyocyte as a preprohormone (preproBNP) of 134 amino acids, from which a prehormone (proBNP) of 108 amino acids and a signal peptide of 26 amino acids is derived. The proBNP precursor protein is then cleaved into a physiologically active 32 amino acid C-terminal peptide (BNP 77 – 108; BNP-32) and a 76 amino acid N-terminal prohormone fragment (NT-proBNP 1 – 76). Studies indicate that the proBNP protein precursor is cleaved either within or on the surface of cardiomyocytes, and that both NT-proBNP (1 – 76) and physiologically active C-terminal BNP-32 molecule (77 – 108) are released into the bloodstream.[11-13]

BNP can be used for a wide range of clinical applications including diagnosis, monitoring and prognosis.[3,4,11,12,14-16] The circulating levels of BNP increase with decreasing left ventricular function and increasing clinical severity of heart failure, according to the NYHA classification, which makes it an appropriate test for diagnosis and staging of heart failure.[17-27] Other studies have demonstrated that an increased level of circulating BNP correlates with higher incidence of cardiac events and mortality in patients with heart failure.[28-31] In patients with Acute Coronary Syndromes, studies have demonstrated that BNP can be used as a marker for patient prognosis.[32,33] The Thrombolysis in Myocardial Infarction 23 (ENTIRETIMI23) Sub-Study evaluated the prognostic performance of the ADVIA Centaur® BNP method assessed at the time of initial presentation in a well-characterized cohort of patients with ST Elevation Myocardial Infarction (STEMI) patients who had an episode of ischemic discomfort of at least 30 minutes duration within the prior 6 hours.[34] This study found that BNP levels greater than 80 pg/mL at presentation were associated with a substantially higher risk of death within the 30-day follow-up period. Additionally, the A to Z study was an international trial of 4266 patients with either non-ST-elevation ACS or ST-elevation MI where ADVIA Centaur® BNP concentration was measured at presentation. Patients with baseline BNP levels > 80 pg/mL had significantly higher two-year risk of death, new-onset heart failure, or combined risk of death or new-onset heart failure.[35] There are also indications that BNP can be used to provide an index to modulate treatment of patients with heart failure.[16,17,36,37]

It has been reported that patients with acute decompensated heart failure who are candidates for nesiritide (recombinant BNP) infusion should have a baseline BNP measurement taken prior to the initiation of therapy. Measurements taken during infusion are reflective of the dose of nesiritide.[38] Because of the short half-life of BNP (20 minutes), measurements taken 2 hours after the cessation of treatment again reflect the level of endogenous BNP. It has also been reported that following infusion, endogenous BNP levels return to baseline by 1 – 2 hours and continue to drop at 6 hours to about 80% of preinfusion levels, suggesting a resetting of the neuro-hormonal axis and improvement in ventricular wall tension as a result of treatment.[39] The Dimension Vista® LOCI BNP method is not approved for nesiritide monitoring.

Principles of Procedure: The LOCI BNP method is a homogeneous, sandwich chemiluminescent immunoassay based on LOCI® technology. The LOCI® reagents include two synthetic bead reagents and a biotinylated anti-BNP monoclonal

antibody fragment. The first bead reagent (Chemibeads) is coated with an anti-BNP monoclonal antibody and contains a chemiluminescent dye. The second bead reagent (Sensibeads) is coated with streptavidin and contains a photosensitizer dye. Sample is incubated with biotinylated antibody and Chemibeads to form bead-BNP-biotinylated antibody sandwiches. Sensibeads are added and bind to the biotin to form bead-pair immunocomplexes. Illumination of the complex at 680 nm generates singlet oxygen from Sensibeads which diffuses into the Chemibeads, triggering a chemiluminescent reaction. The resulting signal is measured at 612 nm and is a direct function of the BNP concentration in the sample.[40,41]

Reagents

Wells[a,b]	Form	Ingredient	Concentration[c]	Source
1, 2	Liquid	BNP biotinylated antibody	35 µg/mL	Mouse monoclonal
3, 4	Liquid	BNP Chemibeads	50 µg/mL	Mouse monoclonal
7, 8	Liquid	Streptavidin Sensibeads	1400 µg/mL	Recombinant *E. coli*
9 – 12	Liquid	Assay Buffer		

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Wells 1 – 12 contain buffers, stabilizers and preservatives.
c. Nominal value per well in a cartridge.

Risk and Safety:

H317
P280, P272, P302 + P352, P333 + P313, P501

Warning!

May cause an allergic skin reaction.

Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents and container in accordance with all local, regional, and national regulations.

Contains: 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone.

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

Precautions: Used LOCI® reaction vessels contain human body fluids; handle with appropriate care to avoid skin contact or ingestion.

For *in vitro* diagnostic use.

Reagent Preparation: All reagents are liquid and ready to use.

Store at: 2 – 8°C

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed wells on the instrument are stable for 30 days.

Open Well Stability: 5 days for wells 1 – 12

Specimen Collection and Handling: Recommended specimen type: EDTA plasma

The LOCI BNP method has been evaluated with plasma using EDTA as the anticoagulant. Serum, sodium citrate, lithium heparin, and sodium fluoride sample tubes have also been tested and are not recommended.

Samples and controls stabilized with sodium azide cannot be used.
Plasma can be collected using recommended procedures for collection of diagnostic blood specimens by venipuncture.[42, 43]

Plastic blood collection tubes are recommended for sample collection, as the BNP analyte is unstable in glass containers. Use of glass tubes and transfer pipettes affects accurate quantitation of BNP.[44,45]

Follow the instructions provided with your specimen collection device for use and processing.[46]

For uncentrifuged EDTA whole blood, samples are stable up to 8 hours at ambient temperature (20°C to 25°C) and 12 hours at refrigerated temperature (2°C to 8°C). The average percentage of BNP recovery in uncentrifuged EDTA whole blood after 8 hours at ambient temperature was 89.0% with a range of 67.4% to 96.4% and after 12 hours refrigerated was 91.3% with a range of 82.1% to 95.9%.

After centrifugation, separated EDTA plasma samples are stable up to 8 hours at ambient temperature (20°C to 25°C) and 12 hours at refrigerated temperature (2°C to 8°C). The average percentage of BNP recovery in separated EDTA plasma after

8 hours at ambient temperature was 94.9% with a range of 91.9% to 97.7% and after 12 hours refrigerated was 90.9% with a range of 70.7% to 96.0%.

If separated EDTA plasma samples are not tested within 12 hours, store samples in plastic tubes and freeze at or below -20°C in non-frost free freezers. Samples may undergo up to 4 freeze/thaw cycles without degradation. Samples are stable up to 9 months when stored frozen (at or below -20°C).[47]

Mix thawed samples thoroughly and store at 2°C to 8°C until use. Thawed frozen specimens which are turbid must be clarified by centrifugation prior to testing. Samples should be tested within 8 hours after thawing.

The purpose of specimen handling and storage information is to provide guidance to users; however, users may validate their own procedures for handling and storing patient samples.

Procedure

Materials Provided

LOCI BNP Flex® reagent cartridge, Cat. No. K6424

Materials Required But Not Provided

BNP CAL, Cat. No. KC677
MULTI 2 SDIL, Cat. No. KD694
Quality Control Materials

Test Steps

Sampling, reagent delivery, mixing, and processing are automatically performed by the Dimension Vista® System. For details of this processing, refer to your Dimension Vista® Operator's Guide.

Test Conditions

Sample Volume (delivered to the vessel)	15 µL
BNP biotinylated antibody Volume	20 µL
BNP Chemibeads Volume	20 µL
Streptavidin Sensibeads Volume	15 µL
Temperature	37.0°C
Reaction time	10 minutes
Wavelength	680 nm Illumination, 612 nm Emission
Type of Measurement	Chemiluminescence

Calibration

Calibration Material	BNP CAL, Cat. No. KC677
Calibration Scheme	5 levels, n = 2
Units	pg/mL [pmol/L][d] (pg/mL x 0.289) = [pmol/L]
Typical Calibration Levels	Level 1 (Calibrator A): 0 pg/mL [0 pmol/L] Level 2 (Calibrator B): 100 pg/mL [29 pmol/L] Level 3 (Calibrator C): 1000 pg/mL [290 pmol/L] Level 4 (Calibrator D): 2500 pg/mL [725 pmol/L] Level 5 (Calibrator E): 5250 pg/mL [1520 pmol/L]
Calibration Frequency	Every 30 days for any one lot Calibration interval may be extended based on acceptable verification of calibration.
A new calibration is required:	• For each new lot of Flex® reagent cartridges. • After major maintenance or service, if indicated by quality control results.

- As indicated in laboratory quality control procedures
- When required by government regulations

d. Système International d'Unités [SI Units] are in brackets.

Quality Control

Follow government regulations or accreditation requirements for quality control frequency. At least once each day of use, analyze two levels of a Quality Control (QC) material with known B-type natriuretic peptide concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.

Results: The instrument calculates the concentration of B-type natriuretic peptide in pg/mL [pmol/L] using the calculation scheme described in your Dimension Vista® System Operator's Guide.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Analytical Measurement Range (AMR): 5 – 5000 pg/mL [1.4 – 1445 pmol/L]

This is the range of analyte values that can be measured directly from the specimen without any dilution or pretreatment that is not part of the usual analytical process and is equivalent to the assay range.

Samples with results in excess of 5000 pg/mL [1445 pmol/L] are reported as "Above Assay Range" and should be repeated on dilution.

Autodilution (AD): Instrument does a 1:2 dilution in the aliquot tray and the normal 15 µL sample volume is still used for over-range samples. Refer to your Dimension Vista® Operator's Guide for more information on the autodilution features.

Manual Dilution: Dilute with MULTI 2 SDIL, Cat. No. KD694 to obtain results within the analytical measurement range. The recommended dilution factor is 1:2. Enter dilution factor on the instrument. Reassay. Result is corrected for dilution.

Note: For manual dilution, time from dilution to measurement is important in obtaining optimum dilution recovery. It is recommended that diluted patient samples be assayed within 30 minutes of dilution preparation using the STAT function.

Samples with results less than 5 pg/mL [1.4 pmol/L] will be reported as "less than 5 pg/mL [1.4 pmol/L]" by the instrument.

Limitations of Procedure

Patient samples may contain heterophilic antibodies that could react in immunoassays to give falsely elevated or depressed results. This method has been designed to minimize interference from heterophilic antibodies. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.[48,49]

The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in LOCI BNP results. Refer to your Dimension Vista® Operator's Guide for the meaning of report flags and comments. Any report containing flags and/or comments should be addressed according to your laboratory's procedure manual and not reported.

Siemens LOCI BNP test results should not be used interchangeably with other manufacturers' BNP methods, nor should Siemens LOCI BNP test results be used interchangeably with NT-proBNP test results.

Measurements of BNP should occur prior to nesiritide (Natrecore) recombinant BNP treatment or 2 hours post-treatment.[50]

Interfering substances

The LOCI BNP method was evaluated for interference according to CLSI/NCCLS EP7-A2.[51] Bias is the difference in the results between the control sample (without the interferent) and the test sample (contains the interferent) expressed in percent. Bias exceeding 10% is considered interference.*

Bilirubin (conjugated) at 40 mg/dL [684 µmol/L] decreases BNP results at a concentration of 82 pg/mL [23.7 pmol/L] by -8.2% and decreases BNP results at a concentration of 628 pg/mL [181 pmol/L] by -12.6%.

Immunoglobulin G (IgG) at 5000 mg/dL [50 g/L] decreases BNP results at a concentration of 64 pg/mL [18.5 pmol/L] by -6.4% and IgG at 4200 mg/dL [42 g/L] decreases BNP results at a concentration of 716 pg/mL [207 pmol/L] by -11%.

Protein: Albumin at 6000 mg/dL [60 g/L] decreases BNP results at a concentration of 58 pg/mL [16.8 pmol/L] by -11.1% and decreases BNP results at a concentration of 646 pg/mL [187 pmol/L] by -12.9%.

*** Analyte results should not be corrected based on this bias.**

Expected Values

LOCI BNP concentrations in the Reference Group are shown in the following tables. Each laboratory should establish its own reference interval for the LOCI BNP method as determined on the Dimension Vista® System. The recommended medical decision threshold is:

Patients 100 pg/mL [28.9 pmol/L]

Reference Study Group

LOCI BNP concentrations were determined in 410 individuals without congestive heart failure (223 women and 187 men). This population included apparently healthy individuals and individuals with diabetes, hypertension, and pulmonary disease. The statistics for LOCI BNP concentrations in the reference study group are shown in the following table.

Reference Study Group

All

	< 45 yrs	**45 – 54 yrs**	**55 – 64 yrs**	**65 – 74 yrs**	**≥ 75 yrs**
Mean	11	13	22	32	80
SD	14	16	36	47	129
Median	5	7	10	17	35
95th Percentile	43	40	81	157	283
% < 100 pg/mL	100	100	97	95	79
N	47	61	87	94	121

Males

	< 45 yrs	**45 – 54 yrs**	**55 – 64 yrs**	**65 – 74 yrs**	**≥ 75 yrs**
Mean	11	16	27	34	107
SD	15	22	47	48	179
Median	5	5	8	23	34
95th Percentile	43	79	103	86	528
% < 100 pg/mL	100	100	94	97	74
N	23	30	49	35	50

Females

	< 45 yrs	**45 – 54 yrs**	**55 – 64 yrs**	**65 – 74 yrs**	**≥ 75 yrs**
Mean	11	11	16	31	61
SD	13	8	11	46	71
Median	5	8	16	12	38
95th Percentile	43	23	42	164	159
% < 100 pg/mL	100	100	100	93	85
N	24	31	38	59	71

Disease Study Group

Blood samples were obtained from 402 patients diagnosed with congestive heart failure (CHF). The population included 167 women and 235 men. The descriptive statistics and New York Heart Association (NYHA) functional classes are provided below. Each laboratory should establish a reference range that represents the patient population that is to be evaluated. In addition, laboratories should be aware of their institution's current practice for the evaluation of CHF.

CHF Population – All

	< 45 yrs	**45 – 54 yrs**	**55 – 64 yrs**	**65 – 74 yrs**	**≥ 75 yrs**
Mean	279	617	490	367	418

SD	347	891	657	419	520
Median	134	278	194	240	267
95th Percentile	1461	1998	1665	1208	1326
% ≥ 100 pg/mL	58	79	64	64	77
N	19	48	81	94	160

Males

	< 45 yrs	**45 – 54 yrs**	**55 – 64 yrs**	**65 – 74 yrs**	**≥ 75 yrs**
Mean	189	724	504	390	424
SD	233	957	570	446	540
Median	120	391	242	274	285
95th Percentile	748	3054	1486	1110	1317
% ≥ 100 pg/mL	58	82	67	67	82
N	12	39	51	52	81

Females

	< 45 yrs	**45 – 54 yrs**	**55 – 64 yrs**	**65 – 74 yrs**	**≥ 75 yrs**
Mean	433	151	467	342	355
SD	467	109	793	387	451
Median	238	102	163	179	205
95th Percentile	1145	1145	2410	1208	1244
% ≥ 100 pg/mL	57	67	60	60	72
N	7	9	30	42	79

CHF Population – All

NYHA Functional Class

	All CHF	**NYHA I**	**NYHA II**	**NYHA III**	**NYHA IV**
Mean	438	186	320	557	713
SD	583	274	369	677	740
Median	232	85	175	342	408
5th Percentile	9	5	9	8	17
95th Percentile	1382	726	1234	1385	2024
% > Cutoff	71	43	71	82	86
Minimum	5	5	5	5	5
Maximum	5000	1495	1816	5000	3510
N	402	96	97	139	70

CHF Population – Males

NYHA Functional Class

	All CHF	NYHA I	NYHA II	NYHA III	NYHA IV
Mean	491	230	378	581	711
SD	627	296	446	755	652
Median	278	99	202	330	548
5th Percentile	6	5	5	8	49
95th Percentile	1461	1110	1317	2447	1998
% > Cutoff	74	49	68	82	91
Minimum	5	5	5	5	5
Maximum	5000	1206	1816	5000	3136
N	235	49	47	96	43

CHF Population – Females

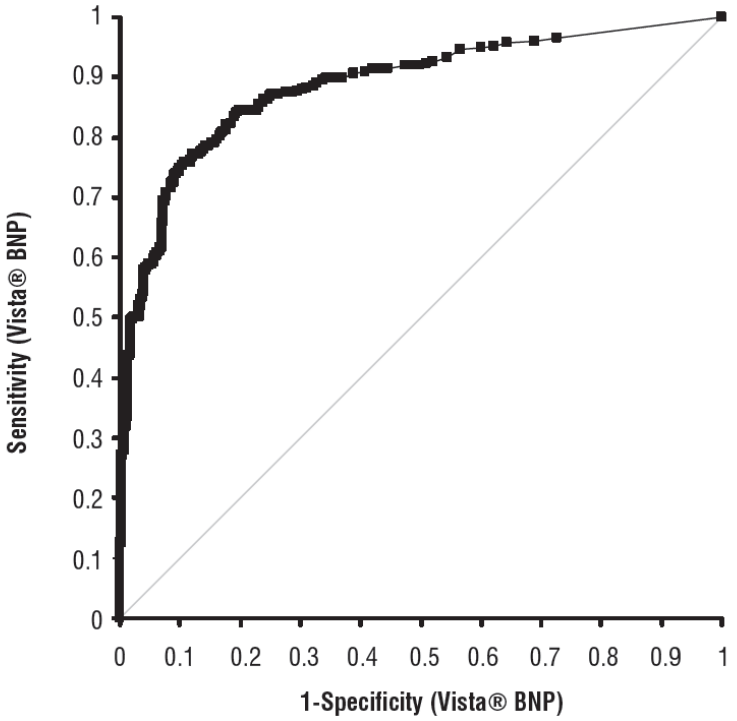
NYHA Functional Class

	All CHF	NYHA I	NYHA II	NYHA III	NYHA IV
Mean	364	139	265	505	717
SD	506	245	273	463	876
Median	168	61	173	486	379
5th Percentile	12	12	12	13	11
95th Percentile	1244	620	800	1145	2686
% > Cutoff	66	36	74	81	78
Minimum	5	5	6	5	5
Maximum	3510	1495	1234	2410	3510
N	167	47	50	43	27

These results show that there is a relationship between the severity of the clinical signs and symptoms of CHF and the median BNP concentration, demonstrating that the Dimension Vista® LOCI BNP method can be used as an aid in the diagnosis of all degrees of CHF severity including asymptomatic patients.

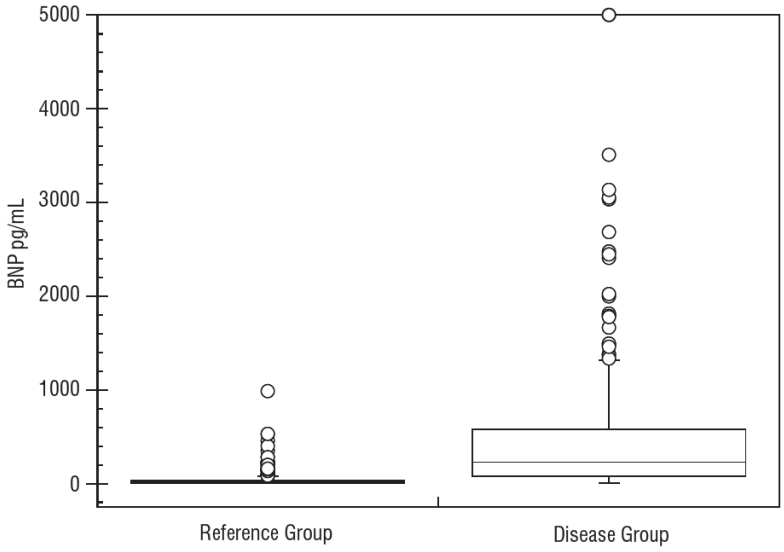
Interpretation of Results

The Receiver Operator Characteristics (ROC) Curve presents the clinical sensitivity and specificity at various cutoffs for the 402 patients diagnosed with CHF and 410 subjects without CHF. The ROC curve for the Dimension Vista® LOCI BNP method is shown below. The area under ROC curve (AUC) for the Dimension Vista® LOCI BNP method is 0.89 with a 95% confidence interval of 0.86 to 0.91.



A box and whiskers plot of the clinical study population is presented below. Recommended clinical threshold is 100 pg/mL [28.9 pmol/L].

Vista® BNP Values for Reference and Disease Groups



Sensitivity and Specificity vs. Age and Gender

The tables below show the clinical sensitivity and specificity of the Dimension Vista® LOCI BNP method using a cutoff of 100 pg/mL [28.9 pmol/L].

Males

	< 45 yrs	**45 – 54 yrs**	**55 – 64 yrs**	**65 – 74 yrs**	**≥ 75 yrs**
Sensitivity	58.3% (7/12)	82.1% (32/39)	66.7% (34/51)	67.3% (35/52)	81.5% (66/81)
95% Confidence Interval	27.8% – 84.8%	66.5% – 92.5%	52.1% – 79.2%	52.9% – 79.7%	71.3% – 89.3%
Specificity	100.0% (23/23)	100.0% (30/30)	93.9% (46/49)	97.1% (34/35)	74.0% (37/50)
95% Confidence Interval	85.2% – 100.0%	88.4% – 100.0%	83.1% – 98.7%	85.1% – 99.9%	59.7% – 85.4%

Females

	< 45 yrs	**45 – 54 yrs**	**55 – 64 yrs**	**65 – 74 yrs**	**≥ 75 yrs**
Sensitivity	57.1% (4/7)	66.7% (6/9)	60.0% (18/30)	59.5% (25/42)	72.2% (57/79)
95% Confidence Interval	18.4% – 90.1%	29.9% – 92.5%	40.6% – 77.3%	43.3% – 74.4%	60.9% – 81.7%
Specificity	100.0% (24/24)	100.0% (31/31)	100.0% (38/38)	93.2% (55/59)	84.5% (60/71)
95% Confidence Interval	85.8% – 100.0%	88.8% – 100.0%	90.8% – 100.0%	83.5% – 98.1%	74.0% – 92.0%

Each laboratory should establish its own expected values for LOCI BNP as performed on the Dimension Vista® System.

Maximum Observed Repeatability

The expected maximum observed standard deviations for repeatability (within-run precision) using n = 5 replicates at the following analyte concentrations are:

LOCI BNP concentration	**Acceptable SD Maximum**
85 pg/mL [25.0 pmol/L]	< 6.2 pg/mL [2.0 pmol/L]
1109 pg/mL [321 pmol/L]	< 33.3 pg/mL [10.0 pmol/L]

A system malfunction may exist if the acceptable SD maximum is exceeded.

Specific Performance Characteristics

The following data represent typical performance for the Dimension Vista® System.

Precision[52,e]

	Mean		**Standard Deviation (%CV)**	
Material	**pg/mL**	**[pmol/L]**	**Repeatability**	**Within-Lab**
Plasma Pool 1	40	[11.6]	1.06 [0.31] (2.7)	1.53 [0.44] (3.9)
Plasma Pool 2	102	[29.5]	1.32 [0.38] (1.3)	3.18 [0.92] (3.1)
Plasma Pool 3	471	[136]	13.71 [3.96] (2.9)	20.05 [5.79] (4.3)
Internal Control 1	1122	[324]	9.22 [2.66] (0.8)	12.77 [3.69] (1.1)
Internal Control 2	3845	[1111]	76.08 [21.99] (2.0)	82.57 [23.86] (2.2)
Cliniqa® Liquid QC™				
Level 3	304	[87.9]	2.75 [0.79] (0.9)	4.14 [1.20] (1.4)

e. CLSI/NCCLS EP5-A2 was used. During each day of testing, two separate runs, with two test samples, for each test material, were analyzed for 20 days.

Cliniqa® and Liquid QC™ are registered trademarks of Cliniqa Corporation, 288 Distribution St., San Marcos, CA 92078, USA.

Method Comparison[53]
Regression Statistics[f]

Comparative Method	Slope	Intercept pg/mL [pmol/L]	Correlation Coefficient	n
ADVIA Centaur® BNP	0.94	2.59 [0.75]	0.991	230[g]

f. CLSI/NCCLS EP9-A2 was used. The method used to fit the regression line was Passing-Bablok.
g. The range of 230 values in the correlation study was 5.3 – 4748 pg/mL [1.53 – 1372 pmol/L].
ADVIA Centaur® is a trademark of Siemens Healthcare Diagnostics.

Specificity

Hemolysis, Icterus, Lipemia (HIL) Interference
The LOCI BNP method was evaluated for interference according to CLSI/NCCLS EP7-A2.[51] Bias is the difference in the results between the control sample (without the interferent) and the test sample (contains the interferent) expressed in percent. Bias exceeding 10% is considered interference.

Substance	Substance Concentration	BNP pg/mL [pmol/L]	Bias** %
Hemoglobin (hemolysate)	500 mg/dL [0.31 mmol/L]	60 [17.3]	≤ 10
		722 [209]	≤ 10
Bilirubin (unconjugated)	60 mg/dL [1026 µmol/L]	76 [22.0]	≤ 10
		579 [167]	≤ 10
Bilirubin (conjugated)	20 mg/dL [342 µmol/L]	52 [15.0]	≤ 10
		556 [161]	≤ 10
Lipemia (Intralipid®)	3000 mg/dL [33.9 mmol/L]	60 [17.3]	≤ 10
		626 [181]	≤ 10

Intralipid® is a registered trademark of Fresenius Kabi AG, Bad Homburg, Germany.
**** Analyte results should not be corrected based on this bias.**

Non-Interfering Substances
The following substances do not interfere with the LOCI BNP method when present in plasma at the concentrations indicated. Inaccuracies (biases) due to these substances are less than 10% at LOCI BNP concentrations of 60 pg/mL [17.3 pmol/L] and 650 pg/mL [188 pmol/L].

Substance	Test Concentration	SI Units
Abciximab	21 µg/mL	441 µmol/L
Acetaminophen	20 mg/dL	1324 µmol/L
Allopurinol	2.5 mg/dL	184 µmol/L
Amidarone	20 µg/mL	31 µmol/L
Amikacin	8.0 mg/dL	137 µmol/L
Amlodipine Besylate	4.0 µg/mL	7.1 µmol/L
Ampicillin	5.3 mg/dL	152 µmol/L
Ascorbic Acid	6.0 mg/dL	342 µmol/L
Atenolol	1.0 mg/dL	37.6 µmol/L
Atorvastatin	32 µg/mL	26.5 µmol/L
Biotin	100 ng/mL	0.41 µmol/L
Caffeine	6.0 mg/dL	308 µmol/L
Carbamazepine	3.0 mg/dL	127 µmol/L
Captopril	5.0 mg/dL	230 µmol/L

Substance	Test Concentration	SI Units
Chloramphenicol	5.0 mg/dL	155 µmol/L
Chlordiazepoxide	1.0 mg/dL	33.3 µmol/L
Chlorpromazine	0.20 mg/dL	6.27 µmol/L
Cholesterol	503 mg/dL	13 mmol/L
Cimetidine	2 mg/dL	79.2 µmol/L
Cinnarizine	3.0 mg/dL	81.4 µmol/L
Clopidogrel bisulfate	30 µg/mL	71.4 µmol/L
Creatinine	30 mg/dL	2.65 mmol/L
Cyclosporine A	4000 ng/mL	3.3 µmol/L
Dextran 40	3000 mg/dL	750 µmol/L
Diazepam	0.51 mg/dL	18 µmol/L
Diclofenac	60 µg/mL	188.6 µmol/L
Digitoxin	60 ng/mL	78.6 nmol/L
Digoxin	6.1 ng/mL	7.8 nmol/L
Diltiazem	120 µg/mL	266.1 µmol/L
Dipyridamole	30 µg/mL	59.4 µmol/L
Dopamine	900,000 pg/mL	5.87 µmol/L
Enalapril maleate	16 µg/mL	32.5 µmol/L
Erythromycin	6.0 mg/dL	81.6 µmol/L
Ethanol	400 mg/dL	86.8 mmol/L
Ethosuximide	25 mg/dL	1770 µmol/L
Furosemide	6.0 mg/dL	181 µmol/L
Gentamicin	1.0 mg/dL	21 µmol/L
Heparin	3.0 U/mL	3000 U/L
Hydralazine	20 µg/mL	101.7 µmol/L
Hydrochlorothiazide	20 µg/mL	67.2 µmol/L
Ibuprofen	50 mg/dL	2425 µmol/L
Immunoglobulin G (IgG)	3300 mg/dL	33 g/L
Indomethacin	36 µg/mL	83.0 µmol/L
Isosorbide dinitrate	4 µg/mL	203 µmol/L
Lidocaine	1.2 mg/dL	51.2 µmol/L
Lithium	2.2 mg/dL	3.2 mmol/L
Lisinopryl	16 µg/mL	36.2 µmol/L
Lovastatin	16 µg/mL	39.6 µmol/L
L-Thyroxine	46 pg/mL	70.84 pmol/L
Methyldopa	100 µg/mL	472 µmol/L
Milrinone	2.4 µg/mL	11.4 µmol/L
Nicotine	0.10 mg/dL	6.2 µmol/L
Nifedipine	36 µg/mL	106 µmol/L
Nitrofurantoin	40 µg/mL	168 µmol/L

Substance	Test Concentration	SI Units
Nitroglycerin	0.16 µg/mL	0.577 µmol/L
Oxazepam	12 µg/mL	41.8 µmol/L
Oxytetracycline	100 µg/mL	217.2 µmol/L
Penicillin G	25 U/mL	25,000 U/L
Pentobarbital	8.0 mg/dL	354 µmol/L
Phenobarbital	10.0 mg/dL	431 µmol/L
Phenytoin	5.0 mg/dL	198 µmol/L
Primidone	4.0 mg/dL	183 µmol/L
Probenecid	600 µg/mL	2103 µmol/L
Propoxyphene	0.16 mg/dL	4.91 µmol/L
Propranolol	64 µg/mL	22 µmol/L
Protein: Albumin	5000 mg/dL	50 g/L
Protein: Total	12000 mg/dL	120 g/L
Quinidine	20 µg/mL	61.6 µmol/L
Salicylic Acid	60 mg/dL	4.34 mmol/L
Simvastatin	32 µg/mL	76.5 µmol/L
Sulfa-methoxazole	400 µg/mL	1579 µmol/L
Theophylline	4.0 mg/dL	222 µmol/L
Triglycerides	3000 mg/dL	33.9 mmol/L
Trimethoprim	64 µg/mL	220.7 µmol/L
Urea	500 mg/dL	83 mmol/L
Uric Acid	20 mg/dL	1.2 mmol/L
Valproic Acid	50 mg/dL	3467 µmol/L
Verapamil	96 µg/mL	0.198 µmol/L
Warfarin	10 µg/mL	32.5 µmol/L

Hook effect

One step sandwich immunoassays are susceptible to a high-dose "hook effect", where an excess of antigen prevents simultaneous binding of the capture and detection antibodies to a single analyte molecule.[54] The LOCI BNP method shows no hook effect up to 100,000 pg/mL [28,900 pmol/L].

Cross-reactivity

Nesiritide at 200 pg/mL cross reacts with BNP results by 15% or less at BNP concentrations of zero and approximately 60 pg/mL [17.3 pmol/L].

Endothelin at 60 pg/mL cross reacts with BNP results by 1.19% at BNP concentrations of zero and approximately 60 pg/mL [17.3 pmol/L].

The following hormones and substances were evaluated for cross-reactivity with the LOCI BNP method and have no significant cross-reactivity (less than 1%) at the concentrations indicated when present in plasma containing 0 pg/mL [0 pmol/L] and 60 pg/mL [17.3 pmol/L] of B-type natriuretic peptide.

Substances	Concentration
Alpha-ANP_{1-28}	1000 pg/mL
NT-$proBNP_{1-21}$	1000 pg/mL
NT-$proBNP_{1-46}$	1000 pg/mL

Substances	Concentration
NT-proBNP$_{1-76}$	1000 pg/mL
NT-proBNP$_{22-46}$	1000 pg/mL
NT-proBNP$_{47-76}$	1000 pg/mL
CNP$_{7-28}$	1000 pg/mL
preproANF$_{104-123}$	1000 pg/mL
preproANF$_{26-55}$	1000 pg/mL
preproANF$_{56-92}$	1000 pg/mL
Adrenomedullin	1 µg/mL
Aldosterone	1000 pg/mL
Angiotensin I	600 pg/mL
Angiotensin II	600 pg/mL
Angiotensin III	1000 pg/mL
Arg-Vasopressin	1000 pg/mL
Renin	50 ng/mL
Urodilatin	1000 pg/mL

Dilution Recovery

Ten human plasma samples in the range of 505 to 6866 pg/mL [146 to 1984 pmol/L] of LOCI BNP were diluted on-board 1:2 with MULTI 2 SDIL and assayed for recovery and parallelism. The recoveries ranged from 95% to 107% with a mean of 101%.

Linearity

Linearity was evaluated by using 7 equally spaced samples prepared by mixing of high and low B-type natriuretic peptide concentration patient plasma samples across the measurement range. Additional patient plasma samples were diluted linearly to confirm dilutional accuracy at the low end of the measurement range. The following table summarizes the results of these studies:

Regression Statistics[h]

Range	Slope	Intercept pg/mL [pmol/L]	Correlation Coefficient	n	Average % Recovery
Low end	1.00	-1.93 [-0.56]	0.999	7	-0.1[i]
Full range	1.06	3.53 [1.02]	0.998	9	-3.1[j]

h. CLSI/NCCLS EP6-A[55] was used. The method used to fit the linear regression line was least squares.
i. The range of BNP values in the low end linearity study was 0 – 651 pg/mL [0 – 188 pmol/L].
j. The range of BNP values in the full range linearity study was 0 – 6036 pg/mL [0 – 1744 pmol/L].

Limit of Detection and Limit of Blank

The Limit of Detection (LoD) for LOCI BNP is 3 pg/mL [0.9 pmol/L], determined consistent with CLSI guideline EP17-A[56] and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 240 determinations, with 4 blank and 4 low level samples. The Limit of Blank (LoB) is 1 pg/mL [0.3 pmol/L].

LoD is the lowest concentration of analyte that can be detected reliably. LoB is the highest concentration that is likely to be observed for a blank sample.

Functional Sensitivity: 10 pg/mL [2.9 pmol/L]

The Functional Sensitivity represents the lowest concentration with an observed 20% coefficient of variation.

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