August 24, 2020

Fujirebio Diagnostics, Inc.
Stacey Dolan
Sr. Manager, Regulatory Affairs
201 Great Valley Parkway
Malvern, PA 19355

Re: K192380
Trade/Device Name: ST AIA-PACK BNP
Regulation Number: 21 CFR 862.1117
Regulation Name: B-Type Natriuretic Peptide Test System
Regulatory Class: Class II
Product Code: NBC
Dated: July 14, 2020
Received: July 15, 2020

Dear Stacey Dolan:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR

803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Marianela Perez-Torres, Ph.D.
Acting Deputy Director
Division of Chemistry
and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
k192380

Device Name
ST AIA-PACK BNP

Indications for Use *(Describe)*
The Tosoh ST AIA-PACK BNP assay is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of BNP in human K2EDTA plasma on Tosoh AIA System analyzers. BNP is used as an aid in the diagnosis of heart failure (HF) in patients presenting to the emergency department (ED) with clinical suspicion of new onset HF, acutely decompensated or exacerbated HF.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Section 5 510(k) SUMMARY

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

A. 510(k) Number:

k192380

B. Purpose for Submission:

New device

C. Measurand:

BNP

D. Type of Test:

Quantitative, Automated Immunoenzymometric assay on the Tosoh AIA System Analyzers

E. Sponsor:

Address: Tosoh Bioscience, Inc.
6000 Shoreline Court, Suite 101
South San Francisco, CA 94080

Contact person: Dave Wurtz
Sr. Director Quality, Regulatory and Clinical
(415) 635-4762
dave.wurtz@tosoh.com

F. Application correspondent:

Address: Fujirebio Diagnostics, Inc.
201 Great Valley Parkway
Malvern, PA 19355

Contact person: Stacey Dolan
Sr. Manager, Regulatory Affairs
(610) 240-3843
dolans@fdi.com

Summary preparation date**:** August 20, 2020

G. Proprietary/Established and Common Names:

1. Proprietary/Established Names:
   ST AIA-PACK BNP

2. Common Name:
   BNP

H. Regulatory Information:

1. Regulation section:
   21 CFR § 862.1117, Test, Natriuretic Peptide

2. Classification:
   Class II

3. Product code:
   NBC, B-Type natriuretic peptide test system

4. Panel:
   75, Chemistry

I. Intended Use:

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

2. Indication(s) for use:

   The Tosoh ST AIA PACK BNP assay is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of BNP in human (K_2EDTA) plasma on Tosoh AIA System Analyzers. BNP is used as an aid in the diagnosis of heart failure (HF) in patients presenting to the emergency department (ED) with clinical suspicion of new onset HF, acutely decompensated or exacerbated HF.

3. Special conditions for use statement(s):
   Prescription use only

4. Special instrument requirements:
   Tosoh AIA System Analyzers

J. Device Description:

The ST AIA-PACK BNP is a two-site immunoenzymometric assay which is performed entirely in the ST AIA-PACK BNP test cups. BNP present in the test sample is bound with monoclonal antibody immobilized on magnetic beads and enzyme-labeled monoclonal antibody. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the BNP concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using the curve.

ST AIA-PACK BNP (Cat. No. 025228)

The ST AIA-PACK BNP set consists of 5 trays x 20 test cups. Each kit contains plastic test cups containing twelve magnetic lyophilized beads coated with anti-BNP mouse monoclonal antibody and 100 µL of anti-BNP mouse monoclonal antibody conjugated to alkaline phosphatase with sodium azide as a preservative.

Other Materials/Equipment Required (not Provided):

AIA Systems:
AIA-2000 ST
AIA-2000 LA

AIA PACK
AIA-PACK Substrate Set II
AIA-PACK Substrate Reagent II/
AIA-PACK Substrate Reconstituent II
AIA-PACK Wash Concentrate
AIA-PACK Diluent Concentrate
Sample Cups
AIA-PACK Detector Standardization Test Cups
Pipette Tips (1000/pkg)
Tip Rack (empty)
Preloaded Pipette Tips (96 tips x 50 racks)
Preloaded Pipette Tips (96 Tips x 5 Racks)

K. Substantial Equivalence Information:

1. Predicate device name(s):
   SIEMENS ADVIA Centaur® BNP Assay

2. Predicate 510(k) number(s):
   K031038

3. Comparison with predicate:

Similarities		
	ST AIA-PACK BNP (Proposed Device)	**SIEMENS ADVIA Centaur® BNP (Predicate Device) K031038**
Device Type	*In vitro* diagnostic	*In vitro* diagnostic
Classification	Class II	Class II
CFR section	21 CFR 862.1117	21 CFR 862.1117
Product Code	NBC	NBC
Product Usage	Clinical and Hospital laboratories	Clinical and Hospital laboratories
Intended Use	The Tosoh ST AIA PACK BNP assay is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of BNP in human (K_2EDTA) plasma on Tosoh AIA System Analyzers. BNP is used as an aid in the diagnosis of heart failure in patients presenting	For in vitro diagnostic use in the quantitative determination of B-type Natriuretic Peptide (BNP) in human plasma using the ADVIA Centaur and ADVIA Centaur XP systems. This assay is indicated for the measurement of plasma BNP as an aid in the diagnosis

Similarities		
	ST AIA-PACK BNP (Proposed Device)	**SIEMENS ADVIA Centaur® BNP (Predicate Device) K031038**
	to the emergency department (ED) with symptoms suggestive of heart failure.	and assessment of the severity of heart failure.
Type of Specimen	Human K_2EDTA plasma	Human EDTA Plasma
Specimen Collection Method	Routine Phlebotomy Techniques	Routine Phlebotomy Techniques
Analyte	Human B-type Natriuretic Peptide (BNP)	Human B-type Natriuretic Peptide (BNP)
Cut-off	100 pg/mL	100 pg/mL

Differences		
	ST AIA-PACK BNP (Proposed Device)	**SIEMENS ADVIA Centaur® BNP (Predicate Device) K031038**
Instrument System	Tosoh AIA Analyzer 2000	ADVIA Centaur and ADVIA Centaur XP Systems
Principle of Operation	Immunoenzymometric Assay	Chemiluminescence immunoassay
Assay Range	4.0 – 2000pg/mL	<2.0 – 5000pg/mL
Detection	Fluorescence	Chemiluminescence
Labeled antibody	Alkaline phosphatase labeled mouse monoclonal (mAb KY-BNP-II) anti-human BNP (Fab')2 fragment specific to the ring structure of BNP. Labeling was done by introducing a malemide group at the hinge portion of the Fab'2 fragment and reacting it with alkaline phosphatase.	Acridinium ester labeled mouse monoclonal (mAb KY-BNP-II) anti-human BNP (Fab')2 fragment specific to the ring structure of BNP.
Solid phase antibody	Mouse monoclonal anti-human BNP (intact) antibody (mAb BC-203) specific to the C-terminal portion of BNP, which is immobilized on magnetic beads	Biotinylated monoclonal mouse anti-human antibody (mAb BC-203) specific to the C-terminal portion of BNP which is coupled to streptavidin magnetic particles
Test principle	One-step sandwich assay	Delayed one-step sandwich assay
Incubation time	~ 10 minutes at 37^0 C	First – 5 minutes at 37^0 C Second – 2.5 minutes at 37^0 C
Reaction	Unbound enzyme-labeled monoclonal antibody is washed away and a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP) is added. The degree of fluorescence is directly	Following the second incubation, the unbound antibody conjugates are washed away. An immune-complex is formed between the BNP in the sample and the two antibody conjugates. The amount of relative light units detected by

	proportional to the amount of BNP in the sample.	the system is directly proportional to the amount of BNP in the sample.

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

- CLSI C28-A3c: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline
- CLSI EP06-A: Evaluation of Linearity of Quantitative Measurement Procedures, A Statistical Approach: Approved Guideline
- CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline
- CLSI EP07: Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition
- CLSI EP37: Supplemental Tables for Interference Testing in Clinical Chemistry; Approved Guideline – First Edition
- CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline
- CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline
- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable; Guidance for Sponsors, Institutional Review Boards, Clinical Investigators, and Food and Drug Administration Staff (April 25, 2006)
- Class II Special Control Guidance Document for B-Type Natriuretic Peptide Premarket Notifications; Final Guidance for Industry and FDA Reviewers. Document issued on: November 30, 2000

M. Test Principle:

The ST AIA-PACK BNP is a two-site immunoenzymometric assay which is performed entirely in the ST AIA-PACK BNP test cups. BNP present in the test sample is bound with monoclonal antibody immobilized on magnetic beads and enzyme-labeled monoclonal antibody. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methyllumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the BNP concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

N. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. *Precision/Reproducibility:*

CLSI Guideline EP5-A2 entitled: "Evaluation of Precision Performance of Quantitative measurement Methods; Approved Guideline – Second Edition. was used to design and CLSI Guideline EP05-A3 entitled: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition* was used to analyze the within run, between run, between day, between lot and total precision studies. The study was conducted at 1 site using three (3) different lots of ST AIA-PACK BNP Reagents and Calibrator Sets on three (3) different AIA-2000 analyzers to assess the precision of the ST AIA-PACK BNP assay.

Six (6) K_2EDTA plasma samples were tested. The compositions and concentrations are described below in the table below.

Sample Number	Approximate Concentration (pg/mL)	Sample Composition
K_2EDTA Plasma-1	10	Apparently healthy patient plasma sample
K_2EDTA Plasma-2	50	Apparently healthy patient plasma sample + patient plasma sample expressing elevated BNP
K_2EDTA Plasma-3	100	Apparently healthy patient plasma sample + patient plasma sample expressing elevated BNP
K_2EDTA Plasma-4	500	Apparently healthy patient plasma sample augmented with BNP antigen
K_2EDTA Plasma-5	1000	Apparently healthy patient plasma sample augmented with BNP antigen

Within run, between run, between day and total precision was calculated for each lot combination of ST AIA-PACK BNP Reagents and Calibrator Sets and are summarized in the tables below. In addition, precision was calculated for the combined lots as summarized in the following tables.

ST AIA-PACK BNP Precision Lot 1(n=80)

Sample		K_2EDTA Plasma-1	K_2EDTA Plasma-2	K_2EDTA Plasma-3	K_2EDTA Plasma-4	K_2EDTA Plasma-5
Mean Conc. (pg/mL)		11.383	50.676	108.286	518.967	1059.189
Within Run	SD	0.529	1.534	2.449	9.763	20.342
	%CV	4.6	3.0	2.3	1.9	1.9
Between Run	SD	0.000	0.000	0.000	2.304	12.679
	%CV	0.0	0.0	0.0	0.4	1.2
Between Day	SD	0.000	0.000	0.000	0.000	4.452
	%CV	0.0	0.0	0.0	0.0	0.4
Total	SD	0.529	1.534	2.449	10.031	24.380
	%CV	4.6	3.0	2.3	1.9	2.3

ST AIA-PACK BNP Precision Lot 2 (n=80)

Sample		K_2EDTA Plasma-1	K_2EDTA Plasma-2	K_2EDTA Plasma-3	K_2EDTA Plasma-4	K_2EDTA Plasma-5
Mean Conc. (pg/mL)		10.896	49.919	104.864	495.956	988.208
Within Run	SD	0.609	1.450	1.997	11.611	25.244
	%CV	5.6	2.9	1.9	2.3	2.6
Between Run	SD	0.000	0.307	1.777	7.232	15.589
	%CV	0.0	0.6	1.7	1.5	1.6
Between Day	SD	0.361	0.722	0.541	0.000	5.415
	%CV	3.3	1.4	0.5	0.0	0.5
Total	SD	0.708	1.648	2.728	13.679	30.159
	%CV	6.5	3.3	2.6	2.8	3.1

ST AIA-PACK BNP Precision Lot 3 (n=80)

Sample		K_2EDTA Plasma-1	K_2EDTA Plasma-2	K_2EDTA Plasma-3	K_2EDTA Plasma-4	K_2EDTA Plasma-5
Mean Conc. (pg/mL)		9.486	49.023	107.004	543.364	1104.740
Within Run	**SD**	0.494	1.642	3.016	8.806	18.148
	%CV	5.2	3.3	2.8	1.6	1.6
Between Run	**SD**	0.000	0.000	0.000	6.076	12.407
	%CV	0.0	0.0	0.0	1.1	1.1
Between Day	**SD**	0.120	0.000	0.000	0.000	0.000
	%CV	1.3	0.0	0.0	0.0	0.0
Total	**SD**	0.508	1.642	3.016	10.699	21.984
	%CV	5.4	3.3	2.8	2.0	2.0

ST AIA-PACK BNP Precision Combined Lots (n=240)

Sample ID	Overall Mean (pg/mL)	Source of Variation	Pooled Standard Deviation	%CV
K_2EDTA Plasma-1	10.588	Within Run	0.547	5.2
		Between Run	0.000	0.0
		Between Day	0.217	2.0
		Between Lot	0.982	9.3
		Total	**1.145**	**10.8**
K_2EDTA Plasma-2	49.873	Within Run	1.592	3.2
		Between Run	0.000	0.0
		Between Day	0.219	0.4
		Between Lot	0.807	1.6
		Total	**1.798**	**3.6**
K_2EDTA Plasma-3	106.718	Within Run	2.637	2.5
		Between Run	0.739	0.7
		Between Day	0.000	0.0
		Between Lot	1.700	1.6
		Total	**3.223**	**3.0**
K_2EDTA Plasma-4	519.429	Within Run	10.127	1.9
		Between Run	5.613	1.1
		Between Day	0.000	0.0
		Between Lot	23.664	4.6
		Total	**26.346**	**5.1**
K_2EDTA Plasma-5	1050.712	Within Run	21.451	2.0
		Between Run	13.823	1.3
		Between Day	3.329	0.3
		Between Lot	58.632	5.6
		Total	**64.031**	**6.1**

b. Linearity/assay reportable range:

The CLSI Guideline EP6-A entitled: "Evaluation of the Linearity of Quantitative Measurement Procedures; A Statistical Approach" Approved Guideline was used to design the linearity study. A total of fourteen (14) samples ranging from 3.1 – 2271.5 pg/mL were assayed to determine linearity. The assay has been demonstrated to be linear from 4.0 to 2000 pg/mL.

Dilution No.	Low : High	Expected Value (pg/mL)	Mean (pg/mL)	SD	CV (%)	Recovery (%)
1	Low Undiluted	3.10	3.28	1.19	36.2	105.6
1.18	9.85 : 0.15	35.04	34.61	1.11	3.2	98.8
1.3	9.7 : 0.3	68.50	67.83	1.07	1.6	99.0
1.6	9.4: 0.6	135.88	137.35	1.01	0.7	101.1
2	9 : 1	223.19	226.19	3.28	1.4	101.3
3	8 : 2	453.41	459.57	4.31	0.9	101.4
4	7 : 3	686.42	688.68	12.98	1.9	100.3
5	6 : 4	905.01	915.36	11.64	1.3	101.1
6	5 : 5	1139.99	1164.54	23.01	2.0	102.2
7	4 : 6	1361.66	1387.54	10.51	0.8	101.9
8	3 : 7	1584.01	1619.32	7.13	0.4	102.2
9	2 : 8	1821.03	1842.89	18.47	1.0	101.2
10	1 : 9	2044.07	2072.97	33.01	1.6	101.4
11	High Undiluted	2271.54	2289.36	20.47	0.9	100.8

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability:
There is currently no known internationally recognized consensus reference method or reference material for standardization. BNP assay values are expressed as pg/mL. The ST AIA-PACK BNP CALIBRATOR SET contains assigned concentrations of BNP. The assigned value is determined on a lot-by-lot basis and is designed to provide an assay calibration range of 4.0 to 2,000 pg/mL of BNP. The calibrators in this set are prepared gravimetrically and are compared to internal reference standards.

Stability:
The stability data supports the current shelf life assignment for the ST AIA-PACK BNP of 12 months.

Expected Values:

d. Detection limit:

The CLSI Guideline EP17-A entitled: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline was used to design the LoD and LoQ study.

Eleven low level samples selected from concentrations in the range from 1.341 to 17.009 pg/mL (LoB to 4x LoB) were prepared by dilution of specimens with known BNP concentrations. The samples were assayed in replicates of two (2) over five (5) days on one instrument for a total of ten (10) replicates per sample. The standard deviation (SD) and coefficient of variation (CV%) were calculated

LoQ was calculated as the functional sensitivity at 20% CV. To determine the functional sensitivity, a precision profile was plotted using the values of CV% and the mean concentration of the samples in pg/mL from the LoD study.

LoB = 0.9 pg/mL

LoD = 1.9 pg/mL

LoQ = 3.5 pg/mL.

e. Analytical specificity:

Studies were conducted to evaluate the potential interference from the endogenous substance summarized below with the Tosoh ST AIA-PACK BNP assay. CLSI Guideline, EP7-A2 entitled: *Interference Testing in Clinical Chemistry - Approved Guideline*, CLSI Guideline EP07 entitled *Interference Testing in Clinical Chemistry; Approved Guideline* – Third Edition and CLSI Guideline EP37 entitled *Supplemental Tables for Interference Testing in Clinical Chemistry; Approved Guideline* – First Edition were used to design the interference study. K_2EDTA samples with known concentrations of BNP, at approximately 35 pg/mL, 90 pg/mL and 1,000 pg/mL were spiked with varying concentrations of the potential interferents.

The criterion for no interference is +/- 10% recovery of BNP in the known specimen mean concentration.

Hemoglobin up to 130 mg/dL does not interfere with the assay.

Unconjugated (Free) Bilirubin up to 15 mg/dL does not interfere with the assay.

Conjugated Bilirubin up to 19 mg/dL does not interfere with the assay.

Lipemia (represented by triglycerides) up to 1600 mg/dL does not interfere with the assay.

Total Protein (represented by human serum albumin) up to 14 g/dL does not interfere with the assay.

Ascorbic Acid up to 20 mg/dL does not interfere with the assay.

Rheumatoid Factor up to 500 IU/mL does not interfere with the assay.

Human IgG up to 5.3 g/dL does not interfere with the assay.

Cholesterol up to 400 mg/dL does not interfere with the assay.

Creatinine up to 15 mg/dL does not interfere with the assay.

Alkaline Phosphatase up to 2000 U/L does not interfere with the assay.

HAMA IgG up to 500 ng/mL does not interfere with the assay.

Studies were conducted to evaluate the potential interference from various compounds with the Tosoh ST AI-PACK BNP assay. K_2EDTA plasma samples with measurable amounts of BNP were spiked with each cross reactant at a concentration of 50, 600 or 1000 pg/mL. Results are summarized below.

Cross Reactant	Test Level (pg/mL)	% Cross Reactivity
Adrenomedullin 52 Human	1000	0.17
Aldosterone	1000	0.11
Angiotensin I	600	-0.02
Angiotensin II	600	-0.13
Angiotensin III	1000	-0.37
ANP	1000	0.03
Arg^8-Vasopressin	1000	0.02
CNP	1000	0.00
DNP	1000	0.43
Endothelin	1000	-0.08
NT-proBNP	1000	-0.05
Renin	50	0.18
Urodilatin	1000	0.00
VNP	900	1.63

Studies were conducted to evaluate the potential interference from several therapeutics with the Tosoh ST AIA-PACK BNP assay. K_2EDTA samples of known BNP concentrations, targeting 100 pg/mL and 300 – 500 pg/mL were used for the study. The therapeutic agent was spiked into each specimen, and the dilution factor was <5% for each agent. The compound and the observed interference for each therapeutic agent are listed below. The % recovery for each therapeutic interferent was within 100+10% of the control.

Tosoh ST AIA-Pack BNP Therapeutic Interference Results

Compound Name	Test Level	Low Level Target 100 pg/ml % recovery	High Level Target 300-500 pg/ml % recovery
Acetaminophen (4-Acetamidophenol)	220 µg/mL	96	99
Acetylsalicylic Acid	200 µg/mL	100	97
Allopurinol	240 ug/mL	98	101
Amiodarone	4.2 mg/dL	107	97
Amlodipine besylate	4 µg/mL	97	98
Ampicillin	200 µg/mL	100	102
L-Ascorbic Acid	66.2 µg/mL	104	102
Atenolol	40 µg/mL	100	101
Atorvastatin	32 µg/mL	98	105
Biotin	30 µg/mL	100	101
Caffeine	10.8 mg/dL	99	100
Carvedilol	30 µg/mL	103	104

Compound Name	Test Level	Low Level Target 100 pg/ml % recovery	High Level Target 300-500 pg/ml % recovery
Captopril	40 µg/mL	97	95
Chloramphenicol	7.8 mg/dL	107	100
Clopidogrel Bisulfate	30 µg/mL	101	98
Cyclosporine	40 µg/mL	99	100
Diclofenac sodium salt	60 µg/mL	103	98
Digitoxin	60 µg/mL	97	98
Digoxin	0.0039 mg/dL	100	97
(+)-cis-Diltiazem hydrochloride	120 µg/mL	97	101
Dipyridamole	30 µg/mL	96	100
Disopyramide	1.68 mg/dL	101	103
Dobutamine	100 µg/mL	99	99
Dopamine hydrochloride	116 µg/mL	103	98
Enalaprilat dehydrate (hydrolyzed from enalapril maleate)	16 µg/mL	100	103
Erythromycin	13.8 mg/dL	107	97
Fenofibrate	45 µg/mL	99	101
Furosemide	65.9 µg/mL	94	92
Heparin	330 units/dL	101	99
Hydralazine	20 µg/mL	100	100
Hydrochlorothiazide	20 µg/mL	98	99
Ibuprofen	500 µg/mL	106	103
Indomethacin	36 µg/mL	104	100
Isosorbide dinitrate	0.593 mg/dL	101	100
Levothyroxine	0.0429 mg/dL	91	94
Lidocaine	1.5 mg/dL	100	97
Lisinopril x 2H2O	16 µg/mL	96	99
Losartan potassium	59.9 µg/mL	99	101
Lovastatin	0.021 mg/dL	103	102
Methyldopa	100 µg/mL	103	101
(±)-Metoprolol (+)-tartrate salt	12.8 µg/mL	102	100
Naproxen	499 µg/mL	101	102
Nicotine	1.6 µg/mL	102	100
Nicotinic acid	40 µg/mL	99	98
Nifedipine	36 µg/mL	98	100
Nitrofuratoin	40 µg/mL	101	102
Oxazepam	12 µg/mL	100	98
Oxytetracycline	100 µg/mL	99	102
Phenobarbital	69 mg/dL	99	103
Phenytoin	6.00 mg/dL	96	103

Compound Name	Test Level	Low Level Target 100 pg/ml % recovery	High Level Target 300-500 pg/ml % recovery
Probenecid	600 µg/mL	100	98
Procainamide	4.80 mg/dL	96	109
Propanolol	64 µg/mL	102	102
Quinidine	20 µg/mL	99	102
Ramipril	6 µg/mL	101	102
Simvastatin	32 µg/mL	99	102
Spironolactone	600 µg/mL	101	100
Sulfamethoxazole	0.43 µg/mL	95	103
Theophylline	6.00 mg/dL	101	101
Trymethoprim	64 µg/mL	99	100
Verapamil hydrochloride	96 µg/mL	100	105
Warfarin	7.5 mg/dL	109	100
Trasylol/Aprotinin	100 KIE/mL	97	100

f. Assay cut-off:

See Clinical Cutoff in M (5) below

2. Comparison studies:
N/A

3. Clinical studies:

This study was conducted to determine the performance of the ST AIA-PACK BNP assay at the current cutoff of BNP at 100 pg/mL as recommended in the American Heart Association guidelines for acute heart failure (HF). Additional analysis was conducted to determine performance of the assay by gender and age. Performance was analyzed separately among patients with and without comorbidities. Statistics were performed between these sub-groups to determine if there were any significant differences in performance.

The prospective study enrolled male and female patients from 8 clinical sites comprised of Emergency Departments (ED). The study included patients who were presented to the ED with clinical suspicion of new onset HF, acutely decompensated or exacerbated HF, where a clinician would order a BNP test as part of a differential diagnosis. Patient samples were stored at -20ºC or colder then sent to a central location for testing at a Tosoh Bioscience lab. A total of 825 samples were assayed for BNP using the Tosoh AIA 2000 Analyzer. Out of the 825 samples, a total number of 724 samples were used for the analysis. 101 samples were excluded from the analysis. Most of these exclusions were due to hemolysis and patients with severe renal insufficiency requiring dialysis (estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73m2): Chronic Kidney Disease (CKD) Stage 4 and 5.

The participants in this study were categorized into the following races: White (n=483, 66.7%), Black (n=144, 19.9%) and other (n=97, 13.3%). The population tested was fairly balanced with respect to females (n=327) versus males (n=397) and older (age ≥ 75 years, n=247) versus younger (age < 75 years, n= 477) subjects. Below is the Age Demographics for Evaluable Subjects.

Summary of Age Demographics for Evaluable Subjects

Age Group (years)	All
22-29	11
30-39	28
40-49	55
50-59	140
60-69	159
70-79	155
80-89	136
≥ 90	40
Total	**724**
Mean Age	67
Median Age	67
Standard Deviation (SD)	15.3
Minimum Age	24
Maximum Age	97

Diagnosis of HF or non- HF was determined by an independent central adjudication panel in order to ensure standardization and accuracy of diagnosis per 2013 ACCF/AHA Guidelines for Management of HF.

The adjudication panel (comprised of 4 expert cardiologists and 1 ER physician) had access to patient CRFs and clinical information, including but not limited to, echocardiography, and other cardiac and thoracic imaging, and standard of care BNP or B-type Natriuretic Peptide, N-terminal Pro B-type Natriuretic Peptide (NT-proBNP) results, if available.

The adjudication panel was blinded to the attending physician's final diagnosis and NYHA classification.

Patients with Heart Failure (HF)

Among the 724 subjects who presented to the ED, 329 patients were determined to have HF by the adjudication panel. All of the patients with HF in the study were categorized utilizing the New York Heart Association (NYHA) Classification system. The NYHA is a four-stage assessment tool that classifies the stage of heart failure based on the subjective observation of a patient's clinical signs and symptoms. It is based on the patient's limitations in physical activity, difficulty with regard to breathing, and angina pain. Below is a description of the classification tool.

NYHA Classification - The Stages of Heart Failure:

NYHA Classification	

Class I	No symptoms and no limitation in ordinary physical activity
Class II	Mild shortness of breath and/or angina and slight limitation during ordinary activity
Class III	Marked limitation in activity due to symptoms, even during less- than ordinary activity
Class IV	Severe limitations. Experiences symptoms even while at rest

The tables below reflect the BNP levels for all HF patients stratified by their NYHA classification.

Heart Failure Population: All

	All HF	NYHA I	NYHA II	NYHA III	NYHA IV
Sample size (N=)	329	2	51	177	99
Mean(pg/mL)		119.0	323.1	673.4	565.9
SD (pg/mL)		69.6	353.4	1074.6	518.8
Median (pg/mL)		119.0	214.7	449.0	420.6
95th percentile		163.0	1124.0	1814.0	1418.0

The BNP cutoff of 100 pg/mL is recommended in the American Heart Association guidelines for acute heart failure (HF). BNP level increase is positively correlated with the severity of heart failure.

The cross tabulation of results between HF and BNP at the cutoff of 100 pg/mL is below.

Cross-tabulation of Patients by Current cutoff: ST AIA PACK BNP Assay

Adjudicated	BNP		
	≥100 pg/mL	<100 pg/mL	Total
HF	291	38	329
Not HF	116	279	395
Total	407	317	724

Using the traditional single cutoff of 100 pg/mL, the sensitivity of the ST AIA-PACK BNP assay is 88.4% and the specificity is 70.6%. The Positive Predictive Value (PPV) is 71.5% and the Negative Predictive Value (NPV) is 88.0%. The prevalence of HF was 45.4% and of no HF was 54.6%

Overall Performance of ST AIA-PACK BNP Assay

Measure	Value	Low CI*	High CI*

Sensitivity	88.4%	84.5%	91.5%
Specificity	70.6%	66.0%	74.9%
Sensitivity + Specificity	159.1%	153.0%	164.3%
Concordance	78.7%	75.6%	81.6%
PPV	71.5%	66.9%	75.7%
NPV	88.0%	84.0%	91.1%
Prevalence of HF	45.4%	41.8%	49.1%
Prevalence of no HF	54.6%	50.9%	58.2%
Positive Likelihood Ratio (PLR), CI	3.012	2.572	3.527
Negative Likelihood Ratio (NLR), CI	0.164	0.120	0.222

*95% CI determined using Wilson Score
Note: subgroup analyses can be found below and may be different than the overall performance.

Receiver Operator Characteristic (ROC)

The utility of BNP as a diagnostic marker for HF is described in multiple reports in the scientific literature. Data from the clinical study was used to generate the Receiver Operating Characteristic (ROC) curve of BNP decision thresholds versus the clinical sensitivity and clinical specificity as shown in Figure 1. The area under the curve (AUC) is 0.881.

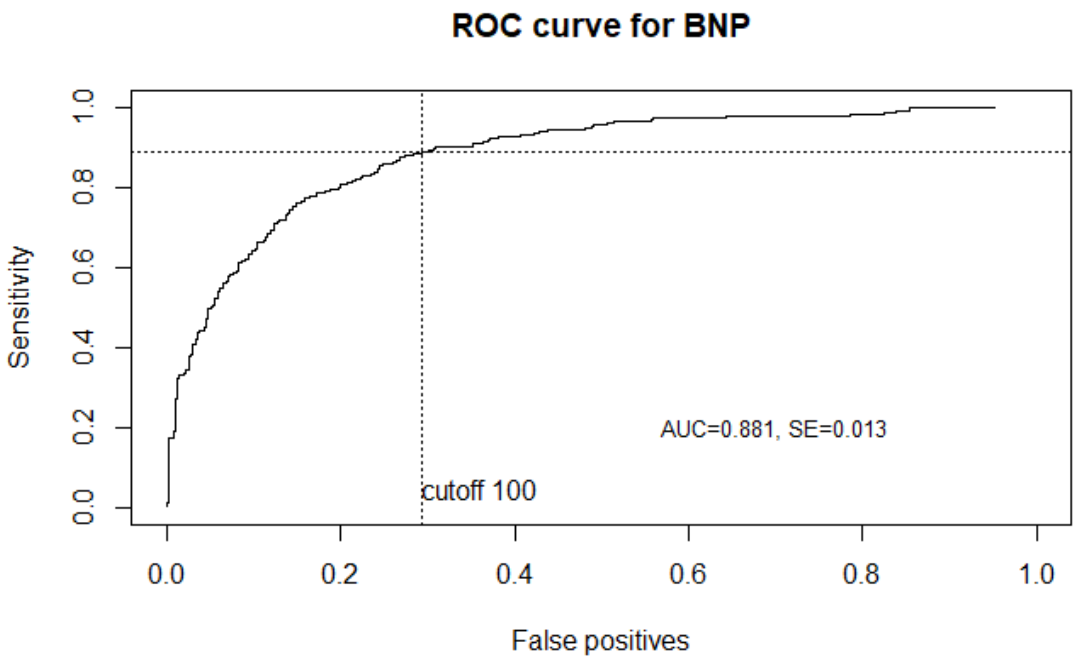

Figure 1. Receiver Operator Curve

4. Clinical cut-off

See M (5) below

5. Expected values/Reference Range

CLSI Guideline C28-A3 entitled: "Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, Approved Guideline" – Third Edition was used to design the reference range study. All specimens were assayed in single replicates utilizing the ST AIA-PACK BNP assay.

To establish the reference range, K_2EDTA plasma samples obtained from apparently healthy males and females were tested using the ST AIA-PACK BNP. The descriptive statistics for BNP concentrations in the apparently healthy (normal) population is shown in the table below.

Reference Group: All

	Age					
	All	**<45**	**45-54**	**55-64**	**65-74**	**≥75**
N	430	92	83	88	87	80
Mean pg/mL	34.6	10.4	11.9	15.1	58.1	81.6
SD pg/mL	73.8	11.0	11.2	18.1	106.6	110.2
Median pg/mL	10.8	5.9	7.9	8.5	14.7	39.1
95 percentile pg/mL	143.2	26.0	39.8	42.5	241.2	273.6
% < 100 pg/mL	91.4	100.0	100.0	98.9	82.8	73.8
Minimum pg/mL	<4	<4	<4	<4	<4	<4
Maximum pg/mL	627.5	80.0	51.6	121.3	619.1	627.5

O. Conclusion

The results of these analytical (nonclinical) and clinical studies demonstrate that the Tosoh ST AIA-PACK BNP assay is substantially equivalent to the performance of the SIEMENS ADVIA Centaur® BNP assay.