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

A. 510(k) Number:

k051596

B. Purpose for Submission:

New device

C. Measurand:

B-type Natriuretic Peptide

D. Type of Test:

Quantitative

E. Applicant:

Nanogen, Inc.

F. Proprietary and Established Names:

*Status*First™ CHF NT-proBNP

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1117

2. Classification:

   Class II

3. Product code:

   NBC

4. Panel:

   75, Chemistry

H. Intended Use:

1. Intended use(s):

   See Indications for Use

2. Indication(s) for use:

   *Status*First™ CHF (Congestive Heart Failure) NT-proBNP is a rapid test for the in-vitro quantitative determination of N-terminal pro-Brain Natriuretic peptide (NT-proBNP) in human EDTA plasma. The device is intended for use with the DXpress™ Reader to provide quantitative results as an aid in the diagnosis of CHF.

3. Special conditions for use statement(s):

   Prescription Use

4. Special instrument requirements:

   Princeton BioMeditech DXpress™ Reader – cleared in k050955

I. Device Description:

Each box contains the following:

- 20 *Status*First™ CHF test devices, each individually sealed in a foil pouch with a dessicant. Each test device contains dye conjugated polyclonal anti-NT-proBNP antibodies, biotin conjugated monoclonal anti-NT-proBNP antibody and streptavidin immobilized at the test band.
- 20 single use droppers
- 1 lot specific Data Chip with calibration information
- 1 package insert

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Roche Elecsys proBNP Immunoassay

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

   k032646

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Expected values (cutoff)	125 pg/mL for patients younger than 75 years and 450 pg/mL for patients 75 years and older	Same

Differences		
Item	Device	Predicate
Principle	Chromatographic immunoassay	Electrochemiluminescence immunoassay
Sample type	EDTA plasma only	Serum and plasma
Instrument	DXpress Reader	Elecsys instruments
Measuring range	20-5000 pg/mL	5-35,000 pg/mL

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

CLSI EP17-A, CLSI EP7-A, CLSI EP5-A, CLSI EP6-A

L. Test Principle:

The *Status*First™ CHF test device utilizes biotin coupled anti-NT-proBNP antibody/streptavidin solid-phase chromatographic immunoassay technology to quantitatively determine the concentration of NT-proBNP in human EDTA plasma specimens. After a sample has been dispensed into the sample well, the *Status*First™ CHF test device is placed in the DXpress™ Reader. The DXpress™ Reader displays the NT-proBNP concentration 15 minutes after sample addition. The DXpress™ Reader is programmed to convert the intensity of the test band (as indicated by the "pBNP" line on the test device) into a concentration of NT-proBNP automatically by using lot specific calibration factors supplied with each box of test devices. The NT-proBNP concentration in the sample correlates with the intensity of the test band.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   The precision of *Status*First™ CHF test device was determined using samples where recombinant NT-proBNP was added at four concentrations. The within

day and total precision was performed in two runs per day, in five replicates per run at each concentration level, for 15 days with three DXpress™ readers. The within-run, total variances and coefficients of variation (CVs) were computed according to CLSI guideline EP5-A. The results are shown below.

Mean level (pg/mL)	Within-run		Total	
	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
64.9	7.19	11.1	8.06	12.4
103.5	13.27	12.8	14.14	13.7
375.5	49.18	13.1	52.18	13.9
2145.8	361.4	16.8	388.0	18.1

b. *Linearity/assay reportable range:*

Each plasma sample having an elevated NT-proBNP concentration (hi pool) was diluted with a sample pool with a low NT-proBNP concentration (<20 pg/mL) for a total of nine values spanning the measuring range of the *Status*First™ CHF test device. Each undiluted and diluted sample was tested in 15 replicates. The results are shown in the table below.

% of high pool	Expected pg/mL	Observed pg/mL	% Recovery
0	N/A	19.46	N/A
0.45	42.6	38.9	91.3
1.25	83.8	74.1	88.4
2.8	163.6	139.2	85.1
7.4	400.3	399.9	99.9
15.1	796.6	791.9	99.4
30.55	1591.7	1548.3	97.3
46	2386.9	2574.9	107.9
61.4	3179.4	3269.7	102.8
76.85	3974.6	4141.5	104.2
100	N/A	5166	N/A

Intercept = 16.0 ng/mL when plotting observed pg/mL versus % of high pool.

The *Status*First™ CHF test has been demonstrated to be linear from 20 pg/mL to 5000 pg/mL, within a 10% deviation from linearity in this interval, calculated in accordance with CLSI Protocol EP6-A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach."

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

Traceable to Roche Elecsys proBNP assay

d. *Detection limit:*

The limit of detection, (LoD), represents the lowest known concentration of NT-proBNP that can be reliably differentiated from zero. The LoD of of the StatusFirst™ CHF test is 20 pg/mL, determined according to Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) guideline EP17-A and with proportions of false positives (α) less than 5% and false negatives (β) less than 5% based on 120 determinations, with 60 blank and 60 low-level samples (limit of blank = 5pg/mL).

e. *Analytical specificity:*

The following proteins and peptides were tested for potential cross-reactivity in the *Status*First™ CHF test device at the maximum concentration of substance indicated. No substance demonstrated significant cross-reactivity (all cross-reactivities < 0.1%) when added to sample containing a recombinant NT-proBNP concentration of approximately 200 pg/mL. The results are shown in the table below.

Substance	Maximum Concentration	Cross-reactivity (%)
BNP-32	1 µg/mL	0.0018
cTnI	3 µg/mL	< 0.001
cTnI/T/C complex	1 µg/mL	< 0.001
CK-MB	3 µg/mL	< 0.001
α-Atrial Natriuretic Polypeptide(α-ANP)(1-28)	1 µg/mL	< 0.001
Prepro-ANP(26-55), ProANP(1-30) Human	1 µg/mL	0.0012
Prepro-ANP(56-92) Human	1 µg/mL	0.0012
Prepro-ANP(104-123), Human	1 µg/mL	0.0016
CNP(C-type natriuretic peptide)	1 µg/mL	0.0022
Urodilatin	0.1 µg/mL	0.0080
Angiotensin I	0.1 µg/mL	0.0192
Angiotensin II	0.1 µg/mL	0.0212
Angiotensin III	0.1 µg/mL	0.0033
Endothelin I	0.1 µg/mL	0.0154
Adrenomedullin (AMD)	0.1 µg/mL	0.0070
Arg-Vasopressin	0.1 µg/mL	<0.001
Renin	0.05 µg/mL	0.0210
Aldosterone	1 µg/mL	0.0013

Sixty-three drugs were assessed for potential interference in the *Status*First™ CHF test device. The list of drugs encompassed common prescription and

over-the-counter compounds, as well as medications often prescribed in a CHF patient population. The drugs were tested at concentrations as recommended in the CLSI Approved Guideline EP7-A 'Interference Testing in Clinical Chemistry', or at least three times the highest concentration reported following a therapeutic dosage. No significant interference with the *Status*First™ CHF measurement was observed for the drugs listed in the table below.

Drug	Drug	Drug
Abciximab	Digoxin	Nitrofurantoin
Acetaminophen	Diltiazem	Nitroglycerin
Acetylsalicylic acid	Dipyridamole	Noramidopyren
Allopurinol	Dopamine	Nystatin
Alteplase	Enalapril maleate	Oxazepam
Ambroxol	Eptafibitide	Oxytetracycline
Amiodarone	Erythromycin	Phenobarbital
Amlodipine Besylate	Fluvastatin	Phenytoin
Ampicillin	Furosemide	Pravastatin
Ascorbic acid (vitamin C)	Glyburide	Probenecid
Atenolol	Heparin	Procainamide
Atorvastatin	Hydralazine	Propranolol
Caffeine	Hydrochlorothiazide	Quinidine
Captopril	Indomethacin	Simvastatin
Chloramphenicol	Isosorbide dinitrate	Spironolactone
Chlordiazepoxide	Lisinopril	Sulfamethoxazole
Cinnarizine	Methaqualone	Theophylline
Clopidogrel bisulphate	Methyl-DOPA	L-thyroxine
Cyclosporine A	Milrinone lactate	Trimethoprim
Diclofenac	Nicotine	Verapamil
Digitoxin	Nifedipine	Warfarin

Other Interfering Substances:

When added to a sample containing NT-proBNP, hemoglobin (up to 0.1 g/dL), bilirubin (up to 10 mg/dL), triglycerides (up to 1.5 g/dL), creatinine (up to 20 µg/mL), and d-biotin (up to 0.1 µg/mL) did not interfere with the recovery of NT-proBNP. No interference was observed from rheumatoid factors (up to 2030 IU/mL) or from high levels of human albumin (up to 16 g/dL).

f. *Assay cut-off:*

The sponsor recommends decision threshold values as follows:
Patients under 75 years of age: 125 pg/mL

Patients 75 years of age and older: 450 pg/mL

NT-proBNP results less than or equal to the decision threshold values are considered normal values representative of patients without CHF

Results greater than the above stated decision threshold values are considered abnormal and suggestive of patients with CHF.

2. Comparison studies:

a. *Method comparison with predicate device:*

A substantial equivalence study was performed between the *Status*First™ CHF and the Roche Elecsys® 2010 proBNP assays using clinical samples within the measuring range of both assays (n=648). When plotting the *Status*First™ results versus the Roche Elecsys® results, the data showed a slope of 0.956 and intercept of 9.4 pg/mL (Passing Bablok regression), and a Spearman Rank correlation of 0.973.

b. *Matrix comparison:*

EDTA plasma is the only sample type indicated

3. Clinical studies:

a. *Clinical Sensitivity:*

Cut off levels of 125 pg/mL for subjects under 75 years of age and 450 pg/mL for subjects over 75 years of age were used to calculate the sensitivity (for CHF subjects) and specificity (for non-CHF subjects) values of the *Status*First™ CHF assay. The results are shown in the table below.

Subjects < 75 years old			Total
*Status*First™ CHF	CHF	Non-CHF	
< 125 pg/mL	17	131	148
≥ 125 pg/mL	199	55	254
Total	216	186	402
Subjects ≥ 75 years old			
*Status*First™ CHF	CHF	Non-CHF	
< 450 pg/mL	17	83	100
≥ 450 pg/mL	122	13	135
Total	139	96	235

The estimates of sensitivities were as follows:

For the subjects less than 75 years of age:
92.1% (199/216)
For the subjects 75 years of age or older:
87.8% (122/139)

The estimates of specificities were as follows:

For the subjects less than 75 years of age:
70.4% (131/186)
For the subjects 75 years of age or older:
86.5% (83/96)

b. *Clinical specificity:*

See Clinical Sensitivity section above.

c. Other clinical supportive data (when a. and b. are not applicable):

Cut off levels of 125pg/mL for subjects under 75 years of age and 450pg/mL for subjects over 75 years of age were used to calculate the sensitivity (for CHF subjects) and specificity (for non-CHF subjects) values of the StatusFirst™ CHF and the Elecsys® proBNP tests. The results are shown in the tables below:

216 CHF subjects < 75 years of age

		Elecsys		
		< 125 pg/mL	≥ 125 pg/mL	
StatusFirst	< 125 pg/mL	13	4	17
	≥ 125 pg/mL	1	198	199
		14	202	216

39 CHF subjects 75[+] years of age

		Elecsys		
		< 450 pg/mL	≥ 450 pg/mL	
StatusFirst	< 450 pg/mL	17	0	17
	≥ 450 pg/mL	5	117	122
		22	117	139

The estimates of sensitivities were as follows:

For the subjects less than 75 years of age:

93.5% (202/216) for Roche Elecsys and 92.1% (199/216) for StatusFirst™;

For the subjects 75 years of age or older:

84.2% (117/139) for Roche Elecsys and 87.8% (122/139) for StatusFirst™.

186 non-CHF subjects < 75 years of age

		Elecsys		
		< 125 pg/mL	≥ 125 pg/mL	
StatusFirst	< 125 pg/mL	124	7	131
	≥ 125 pg/mL	13	42	55
		137	79	186

96 non-CHF subjects 75[+] years of age

		Elecsys		
		< 450 pg/mL	≥ 450 pg/mL	
StatusFirst	< 450 pg/mL	83	0	83
	≥ 450 pg/mL	2	11	13
		85	11	96

The estimates of specificities were as follows:

For the subjects less than 75 years of age:

 73.7% (137/186) for Roche Elecsys and 70.4% (131/186) for StatusFirst™;

For the subjects 75 years of age or older:

88.5% (85/96) for Roche Elecsys and 86.5% (83/96) for StatusFirst™.

Non-CHF subjects (diabetes, renal insufficiency, hypertension or chronic OPD)

12 subjects < 75 years of age

		Elecsys		
		< 125 pg/mL	≥ 125 pg/mL	
StatusFirst	< 125 pg/mL	7	0	7
	≥ 125 pg/mL	0	5	5
		7	5	12

39 subjects 75+ years of age

		Elecsys		
		< 450 pg/mL	≥ 450 pg/mL	
StatusFirst	< 450 pg/mL	18	1	19
	≥ 450 pg/mL	2	18	20
		20	19	39

The estimates of specificities were as follows:

For the subjects less than 75 years of age:

 58.3% (7/12) for Roche Elecsys and 58.3% (7/12) for StatusFirst™;
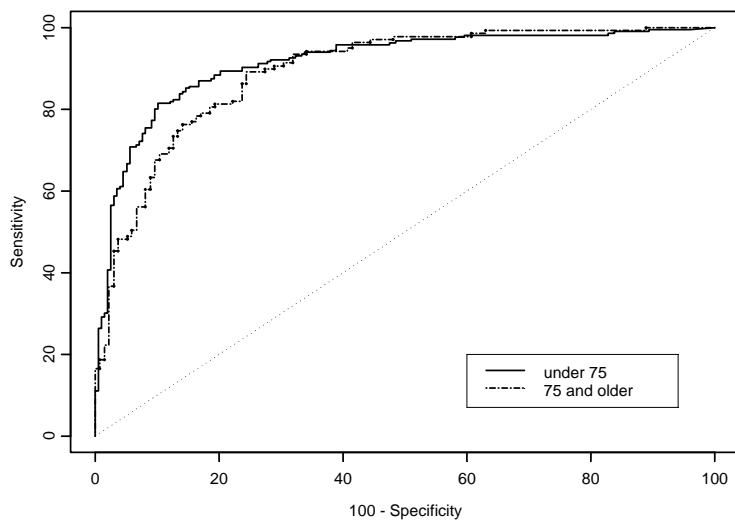
For the subjects 75 years of age or older:

51.3% (20/39) for Roche Elecsys and 48.7% (19/39) for StatusFirst™.

4. Clinical cut-off:

The diagnostic utility of the *Status*First™ CHF test device in CHF patients versus those without CHF is demonstrated by the area under the Receiver Operator Characteristic (ROC) curve of 0.896, which indicates that the *Status*First™ CHF

is effective as an aid in the diagnosis of CHF.

*Status*First™ ROC curves, stratified by age

Subjects less than 75 years old (216 CHF and 198 non-CHF)
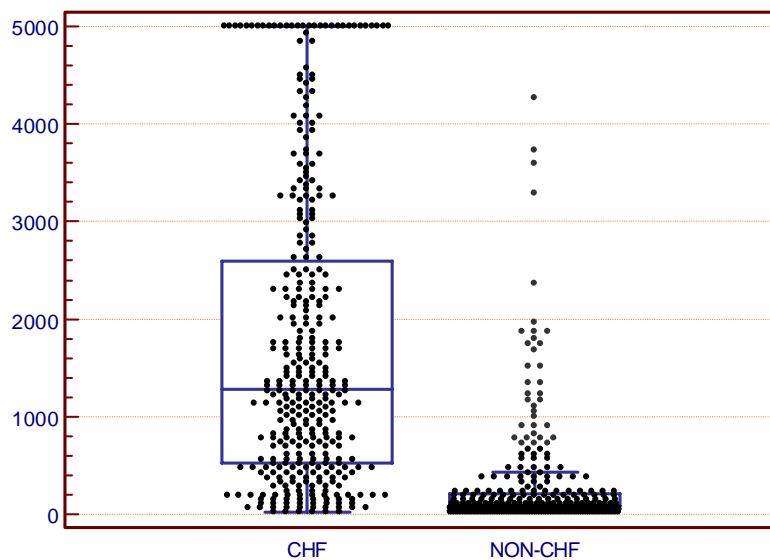Area under Curve (AUC) = 0.915
95% Confidence Interval= [0.884, 0.940]

Subjects 75 years or older (139 CHF and 135 non-CHF),
Area under curve = 0.892
95% Confidence Interval = [0.850, 0.926]

Boxplot of NT-proBNP levels for CHF and non-CHF cohorts

5. Expected values/Reference range:

Individuals without CHF

From a population of 333 individuals without CHF (153 women, 180 men), the *Status*First™ CHF test device was used to determine the concentration of NT-proBNP. This population included apparently healthy individuals and individuals with diabetes, renal insufficiency, hypertension or chronic obstructive pulmonary disease. Summary statistics for NT-proBNP in subjects are given below.

Non-CHF Subjects

	Age Category (years)					
	< 45	45-54	55-64	65-74	75+	< 75
Median	32.6	48.9	55.1	86.0	136.6	71.6
95th percentile	(*)	366.5	217.4	768.8	1850.5	593.0
% < 125 pg/ml	100	76.2	78.0	62.5	-	69.7
% < 450 pg/ml	-	-	-	-	75.6	-
N	6	21	59	112	135	198

(*) Insufficient sample size

Blood samples were obtained from 355 patients diagnosed with CHF (160 women and 195 men). Summary statistics for NT-proBNP concentrations in patients with CHF are presented in the tables below.

CHF Population
*Status*First™ NT-proBNP levels (pg/mL) in males with CHF, stratified by NYHA Class

	NYHA Functional Class				
	All CHF	NYHA I	NYHA II	NYHA III	NYHA IV
Median	1249.6	1052.0	1106.3	1351.0	2763.4
5th percentile	106.2	35.2	133.7	93.7	177.8
95th percentile	> 5000	3570.4	> 5000	> 5000	> 5000
% > cutoff	91.3	87.1	92.9	88.6	95.5
Minimum	< 20	29.0	< 20	59.7	117.1
Maximum	> 5000	3931.6	> 5000	> 5000	> 5000
N	195	31	98	44	22

*Status*First™ NT-proBNP levels (pg/mL) in females with CHF, stratified by NYHA Class

	NYHA Functional Class				
	All CHF	NYHA I	NYHA II	NYHA III	NYHA IV
Median	1316.2	879.8	1588.9	1155.6	1052.1
5th percentile	109.7	73.6	131.7	142.5	N/A
95th percentile	> 5000	2700.2	> 5000	> 5000	> 5000
% > cutoff	89.4	90.5	89.8	86.7	90.9
Minimum	33.5	68.6	33.5	44.3	114.9
Maximum	> 5000	3268.5	> 5000	> 5000	> 5000
N	160	21	98	30	11

*Status*First™ NT-proBNP levels (pg/mL) stratified by age group

	Age Category (years)					
	< 45	45-54	55-64	65-74	75+	< 75
Median	1088.6	599.1	762.5	1265.8	1771.2	1015.6
95th percentile	(*)	1491.2	4096.0	> 5000	> 5000	> 5000
% > 125 pg/ml	87.5	80.0	89.4	95.9	-	92.1
% > 450 pg/ml	-	-	-	-	87.8	-
N	8	20	66	122	139	216

(*) Insufficient sample size

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.