

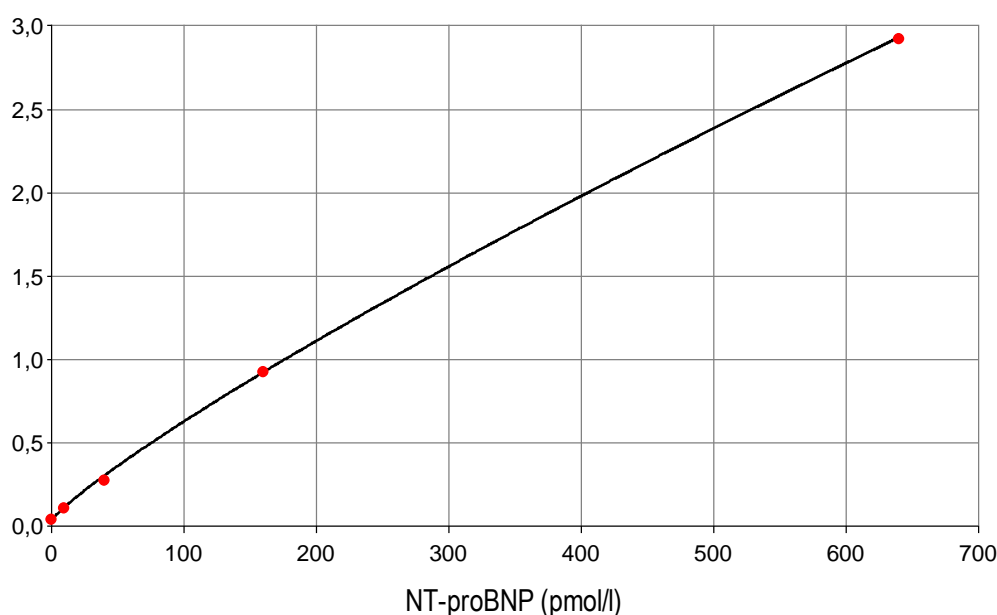
NT-proBNP ELISA (Cat.No. SK-1204)

For the Determination of NT-proBNP (1-76) in Human Samples

ASSAY CHARACTERISTICS

Method	Sandwich ELISA, HRP/TMB, 12x8-well strips
Antibodies/Standard	Capture antibody: polyclonal anti human NT-proBNP antibody, specifically binding to amino acids 31-57 of proBNP. Detection antibody: polyclonal anti human NT-proBNP antibody, conjugated to peroxidase, specifically binding to amino acids 8-29 of proBNP. Standard material: synthetic human NT-proBNP (1-76).
Sample type	Human serum, EDTA plasma
Standard range	0-640 pmol/l (0, 10, 40, 160, 640 pmol/l)
Conversion factor	1 pmol/l = 8.475 pg/ml (MW: 8.475 kDa) <i>refers to NT-proBNP (1-76) that is detected by the ELISA</i>
Sample volume	50 µl / well – direct measurement
Sensitivity	LOD (0pmol/l + 3SD): 3.0 pmol/l; LLOQ: 3.3 pmol/l
Specificity	This assay recognizes endogenous (natural) and recombinant human NT-proBNP (1-76).
Incubation	3 h /30 min – room temperature

Typical standard curve of Biomedica NT-proBNP ELISA:



Serum values from apparently healthy donors:

Serum (n=70)	NT-proBNP (pmol/l)
Median	5.8
Mean	9.8
Maximum	63.9
Minimum	1.1
Percentil 95%	32.5
Percentil 5%	2.0

EDTA plasma values from apparently healthy donors:

EDTA plasma (n=28)	NT-proBNP (pmol/l)
Median	5.6
Mean	9.5
Maximum	32.0
Minimum	0.6
Percentil 95%	27.9
Percentil 5%	0.8

Serum values from unselected hospital panel:

Serum (n=117)	NT-proBNP (pmol/l)
Median	46.5
Mean	84.5
Maximum	621.1
Minimum	0.0
Percentil 95%	22.1
Percentil 5%	0.8

EDTA plasma values from unselected hospital panel:

EDTA plasma (n=40)	NT-proBNP (pmol/l)
Median	28.6
Mean	57.9
Maximum	489.2
Minimum	0.3
Percentil 95%	157.7
Percentil 5%	1.2

Serum values of a heart failure panel NYHA classification II-IV measured with the Biomedica NT-proBNP ELISA (#SK-1204)

Serum (n=66)	NT-proBNP (pmol/l)
mean	47.5
max	399.3
min	1.1

NYHA classification subgroups:

measured with the Biomedica NT-proBNP ELISA (#SK-1204)

Data on serum values of a heart failure panel **NYHA classification II**

Serum (n=43)	NT-proBNP (pmol/l)
mean	24.8
max	75.6
min	1.1

Data on serum values of a heart failure panel **NYHA classification III**

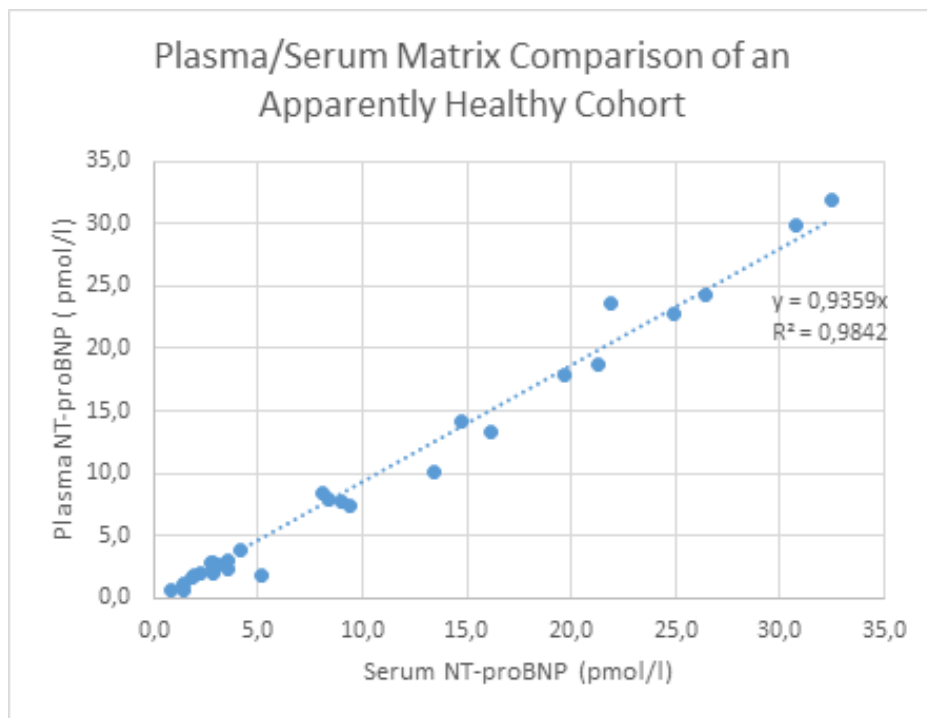
Serum (n=16)	NT-proBNP (pmol/l)
mean	56.2
max	264.3
min	4.7

Data on serum values of a heart failure panel **NYHA classification IV**

Serum (n=7)	NT-proBNP (pmol/l)
mean	166.8
max	399.3
min	84.1

MATRIX COMPARISON

Correlation of serum and plasma samples from apparently healthy individuals



PERFORMANCE CHARACTERISTICS

Spike Recovery in human serum samples:

The mean recovery of recombinant NT-proBNP in serum samples (n=4) when spiked with two different concentrations of human recombinant NT-proBNP is 99 and 108%.

Experiment:

Recovery of spiked samples was tested by adding 2 concentrations of human recombinant NT-proBNP (1-76) to 4 different human serum samples.

Data showing spike/recovery of human serum samples:

Matrix		Serum			S/R (%)	
Spike (pmol/l)		0	80	320	80	320
Sample ID	#1	1.6	88.5	347.2	109	108
	#2	0.1	74.2	360.2	93	113
	#3	1.7	80.9	338.7	99	105
	#4	4.4	81.6	346.5	96	107
Mean (%)					99	108

Spike Recovery in human EDTA plasma samples:

The mean recovery of recombinant NT-proBNP in EDTA plasma samples (n=4) when spiked with two different concentrations of human recombinant NT-proBNP is 93 and 94%.

Experiment:

Recovery of spiked samples was tested by adding 2 concentrations of human recombinant NT-proBNP (1-76) to 4 different human EDTA plasma samples.

Data showing spike/recovery of human EDTA plasma samples:

Matrix		EDTA plasma			S/R (%)	
Spike (pmol/l)		0	80	320	80	320
Sample ID	#1	0.8	73.4	276.8	92	87
	#2	7.7	72.8	279.0	91	87
	#3	9.3	77.2	324.1	97	101
	#4	19.1	74.9	309.5	94	97
Mean (%)					94	93

Dilution Linearity/Parallelism:

Serum (n=3): **mean 117%. Range: 112-119%**
 (samples spiked with recombinant NT-proBNP)
 mean 82%. Range: 79-86% (endogenous NT-proBNP)

EDTA plasma (n=2): **mean 84%. Range: 83-84%**
 (samples spiked with recombinant NT-proBNP)
 mean 84%. Range: 79-80% (endogenous NT-proBNP)

Experiment 1 - Dilution Linearity in human serum samples:

Dilution linearity was assessed by spiking serum samples with recombinant NT-proBNP (1-76) (320 pmol/l) and by diluting the samples with STD1 (serum matrix containing 0 pmol/l NT-proBNP, provided in the kit):

Data showing the dilution of recombinant NT-proBNP (1-76) in serum samples:

Serum Sample ID	Reference NT-proBNP (pmol/l)	Spiked NT-proBNP (pmol/l)	Dilution 1+1	
			NT-proBNP (pmol/l)	R (%)
#1	1.7	338.7	202.2	119
#2	0.1	360.2	202.4	112
#3	8.0	278.5	165.7	119
			Mean (%)	117

Experiment 2 - Parallelism in human serum samples:

Dilution linearity (parallelism) was assessed by diluting serum samples containing endogenous NT-proBNP with STD1 (serum matrix containing 0 pmol/l NT-proBNP, provided in the kit).

Data showing the dilution of endogenous NT-proBNP (1-76) in serum samples:

Serum Sample ID	Reference NT-proBNP (pmol/l)	Dilution 1+1		
		NT-proBNP (pmol/l)	R (%)	
#1	44.2	17.4	79	
#2	51.3	20.4	80	
#3	61.7	26.5	86	
			Mean (%)	82

EDTA plasma (n=2): 83-84% (spiked with recombinant NT-proBNP)
80-85% (endogenous NT-proBNP)

Experiment 1 - Dilution Linearity in human plasma samples:

Dilution linearity was assessed by spiking EDTA plasma samples 1+1 with recombinant NT-proBNP (1-76) (640 pmol/l) and by diluting the samples with STD1 (serum matrix containing 0 pmol/l NT-proBNP, provided in the kit):

Data showing the dilution of recombinant NT-proBNP (1-76) in EDTA plasma samples:

EDTA plasma Sample ID	Reference NT-proBNP (pmol/l)	Spiked NT-proBNP (pmol/l)	Dilution 1+1	
			NT-proBNP (pmol/l)	R (%)
#1	36	347	145	84
#2	0.8	277	115	83
			Mean (%)	84

Experiment 2 – Parallelism in human plasma samples:

Dilution linearity was assessed by diluting EDTA plasma samples containing endogenous NT-proBNP with STD1 (serum matrix containing 0 pmol/l NT-proBNP, provided in the kit).

Data showing the dilution of endogenous NT-proBNP (1-76) in EDTA plasma samples:

EDTA plasma Sample ID	Reference NT-proBNP (pmol/l)	Dilution 1+1	
		NT-proBNP (pmol/l)	R (%)
#1	44.2	17.4	79
#2	51.3	20.4	80
		Mean (%)	84

Intra-assay (With-In Run) & Inter-assay precision (In-Between)

Intra-assay (n=3) ≤ 4%, Inter-assay (n=8) ≤ 7%

Experiment:

Intra-assay: 2 samples of known concentrations were tested 3 times in 1 assay by 1 operator.

Inter-assay: 2 samples of known concentrations were tested 8 times in 2 assays by different operators.

Data showing intra-assay and inter-assay precision:

Intra-assay (n=3)	Sample 1	Sample 2	Inter-assay (n=8)	Sample 1	Sample 2
Mean (pmol/l)	60.2	35.2	Mean (pmol/l)	52.1	108.1
SD (pmol/l)	2.0	0.9	SD (pmol/l)	1.7	7.9
CV (%)	4	3	CV (%)	3	7

The limit of detection (LOD):

The LOD is defined as the mean value of the back calculated concentration plus 3 times the standard deviation. The LOD of the NT-proBNP ELISA is 3 pmol/l.

The lower limit of quantification (LLOQ):

The lower limit of quantification is defined as the accuracy of the back calculated concentrations and shall not exceed ±25% (acc. to ICH (Ref. 1)).

For the NT-proBNP ELISA the LLOQ was determined as 3.3 pmol/l.

Specificity:

This assay recognizes endogenous (natural) and recombinant human NT-proBNP (1-76).

Calibration:

This immunoassay is calibrated against recombinant human NT-proBNP (1-76) peptide.

SAMPLE CHARACTERISTICS

Sample stability:

We recommend separating EDTA plasma or serum by centrifugation as soon as possible, e.g. 20 min at 2,000 x g, preferably at 4°C (2-8°C). EDTA-plasma or serum can be stored at 4°C (2-8°C) up to two days. For long term storage, aliquot the acquired EDTA plasma or serum samples and store them at -25°C or lower.

Samples can be subjected to 3 freeze-thaw cycles.

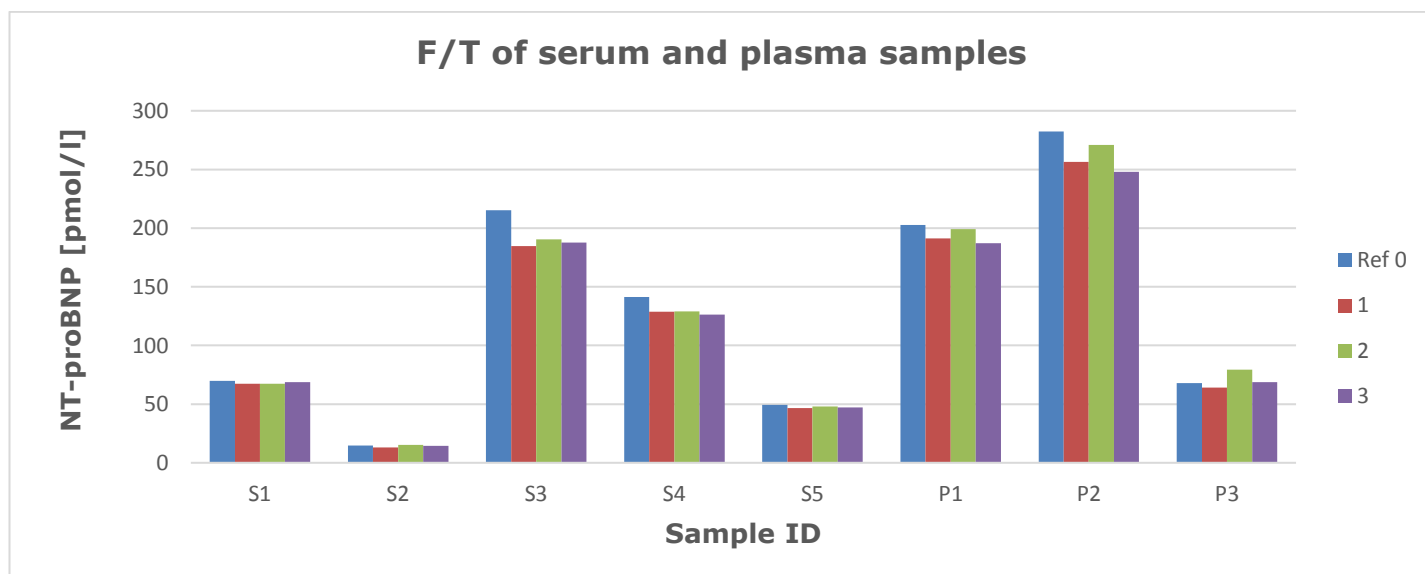
Serum and EDTA samples can be stored for at least 2 years at -80°C.

Freeze/thaw of samples containing endogenous NT-proBNP

The mean recovery of serum and EDTA plasma sample concentrations stressed by 3 F/T cycles is 94%. The mean CV of sample concentrations (not stressed and stressed up to 3 times by freeze-thaw cycles) is 4%.

NT-proBNP concentrations of samples after 3 freeze-thaw cycles:

Sample ID	NT-proBNP [pmol/l]				Mean	CV (%)	R (%) 3 F/T vs ref
	Ref	1	2	3			
Serum1	70	67	67	69	68	1	98
Serum2	15	13	15	14	14	5	98
Serum3	215	185	191	188	194	6	87
Serum4	141	129	129	126	131	4	90
Serum5	49	47	48	47	48	2	96
Plasma1	203	191	199	187	195	3	92
Plasma2	282	257	271	248	264	5	88
Plasma3	68	64	79	69	70	8	101
Mean (%)						4	94



Validation

The assay is fully validated according to ICH Q2 (R1), Ref. (1).

References

(1) CPMP/ICH/381/95 - ICH Topic Q2 (R1) „Validation of Analytical Procedures: Text and Methodology“ including:
ICH Q2A “Text on Validation of Analytical Procedures”
ICH Q2B “Validation of Analytical Procedures: Methodology”

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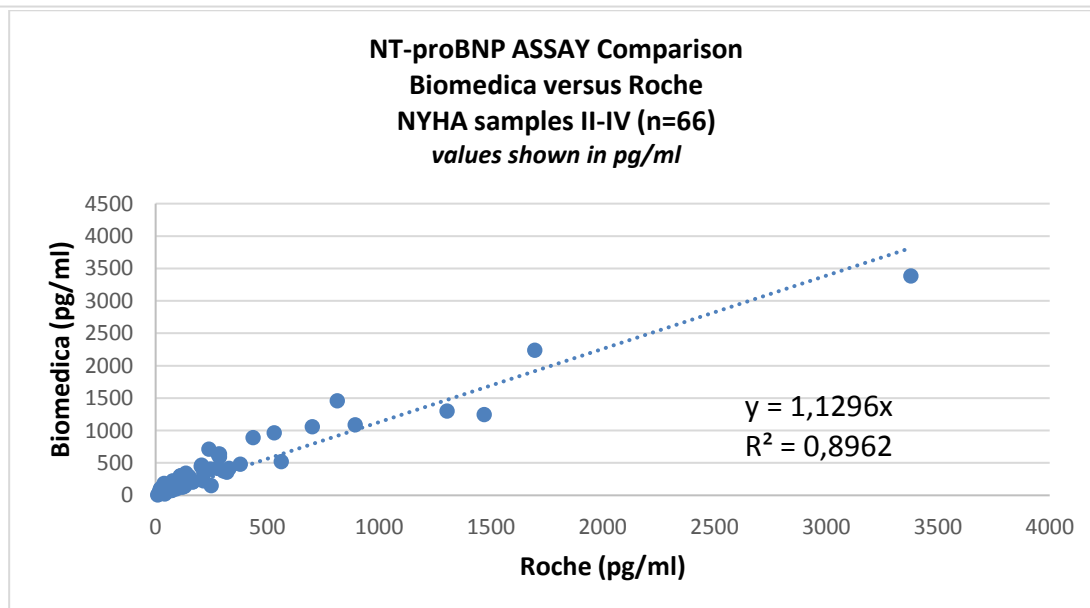
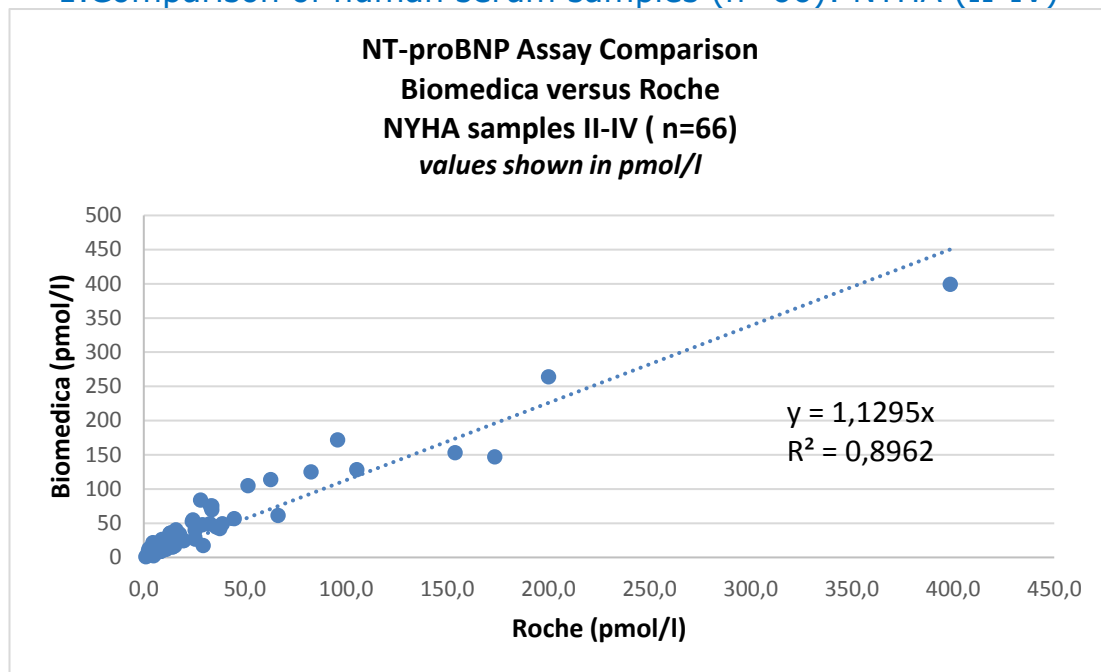
[Enzyme immunoassay for the quantitative determination of NT-proBNP \(1-76\) in human serum \(cat.no. SK-1204\)](#)

Protocol booklet, Validation Data, MSDS, Citations

NT-proBNP ASSAY COMPARISON

Comparison between NT-proBNP Assays: Biomedica #SK-1204 versus Roche Cobas

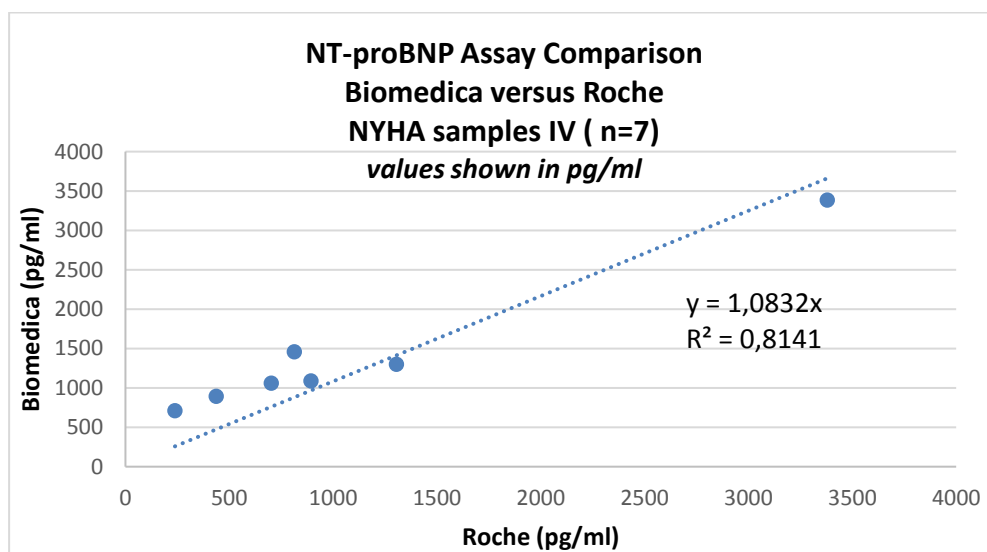
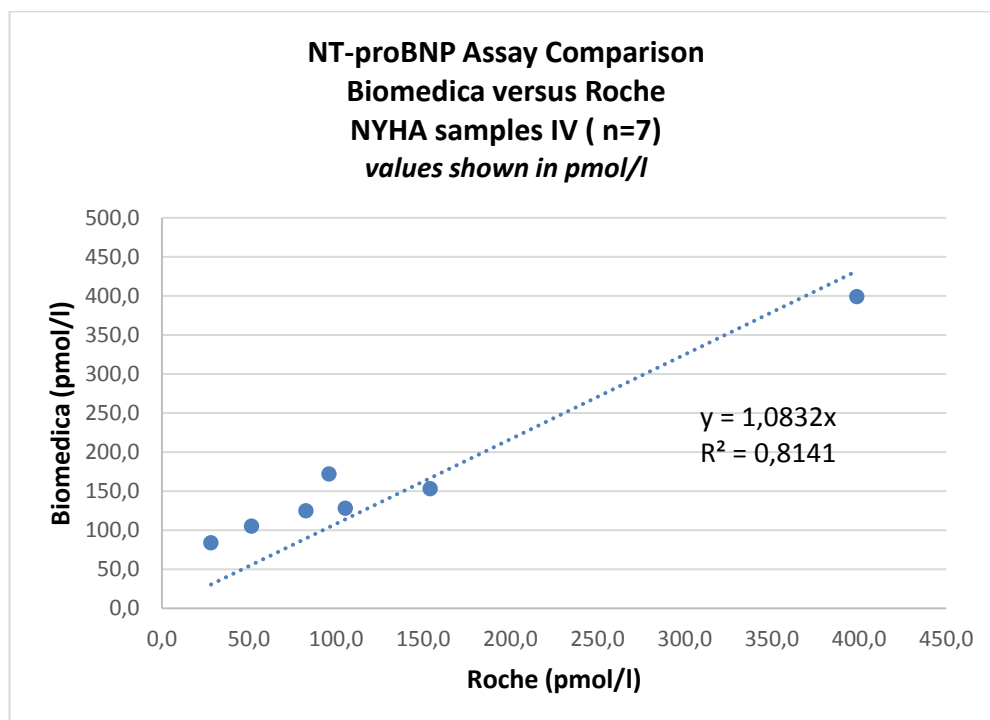
1. Comparison of human serum samples (n=66): NYHA (II-IV)



Comparison of serum values of a heart failure panel **NYHA classification II-IV** measured with the Biomedica NT-proBNP ELISA and the Roche Cobas assay (values shown both in pmol/l and in pg/ml)

n=66	Biomedica <i>pmol/l</i>	Roche <i>pmol/l</i>	Biomedica <i>pg/ml</i>	Roche <i>pg/ml</i>
mean	47.5	33.9	402.5	287.3
max	399.3	398.7	3384.0	3378.9
min	1.1	1.1	9.3	9.3

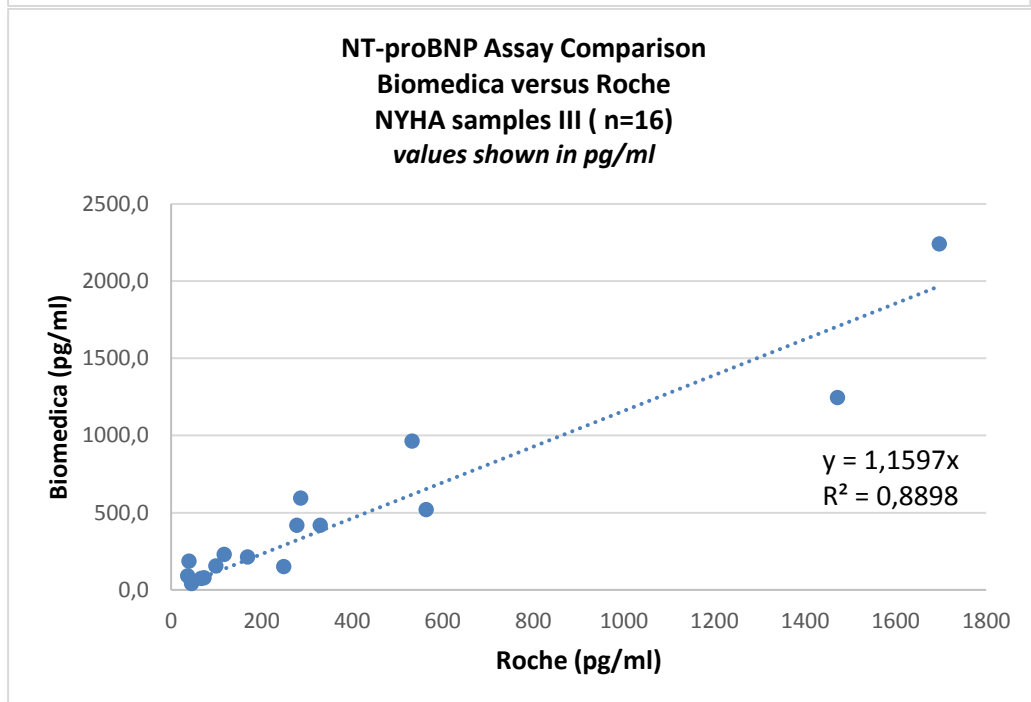
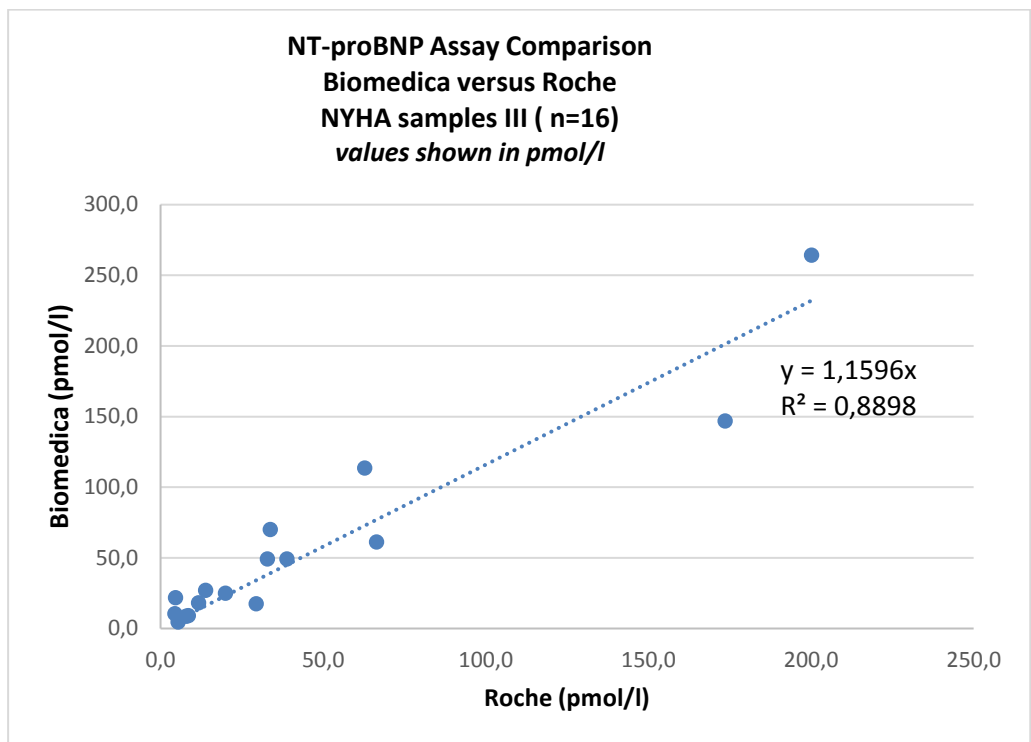
2.Comparison of NYHA (IV) samples (n=7)



Comparison of serum values of a heart failure panel **NYHA classification IV** measured with the Biomedica NT-proBNP ELISA and the Roche Cobas assay (values shown both in pmol/l and in pg/ml)

n=7	Biomedica pmol/l	Roche pmol/l	Biomedica pg/ml	Roche pg/ml
mean	166.8	130.9	1413.6	1109.2
max	399.3	398.7	3384.0	3379.0
min	84.1	28.1	712.7	238.4

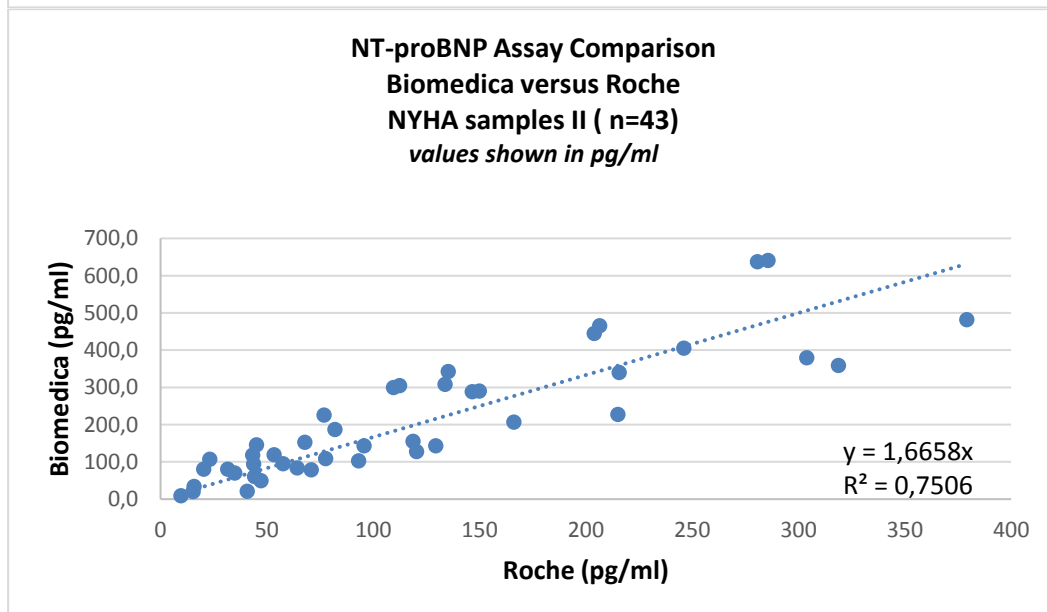
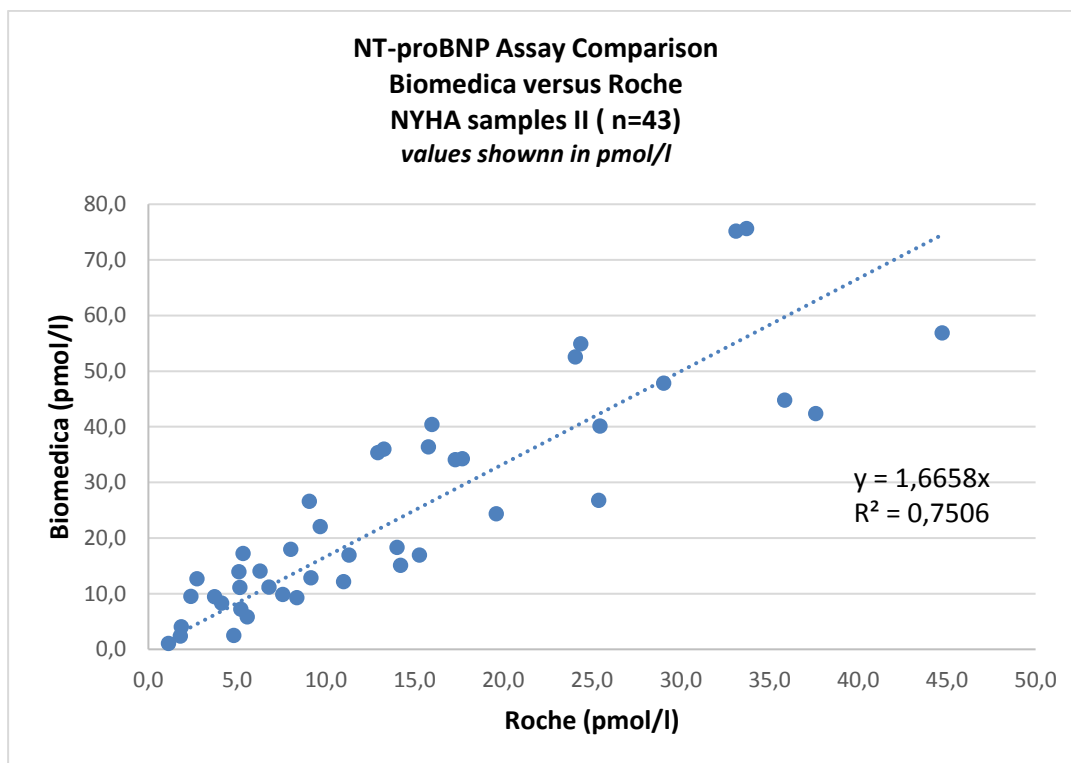
3. Comparison of NYHA (III) samples (n=16)



Comparison of serum values of a heart failure panel **NYHA classification III** measured with the Biomedica NT-proBNP ELISA and the Roche Cobas assay (values shown both in pmol/l and in pg/ml)

n=16	Biomedica pmol/l	Roche pmol/l	Biomedica pg/ml	Roche pg/ml
mean	56.2	44.5	476.2	377.9
max	264.3	200.1	2239.9	1696.0
min	4.7	4.3	39.8	36.6

4. Comparison of NYHA (II) samples (n=43)



Comparison of serum values of a heart failure panel **NYHA classification II** measured with the Biomedica NT-proBNP ELISA and the Roche Cobas assay (values shown both in pmol/l and in pg/ml)

n=43	Biomedica pmol/l	Roche pmol/l	Biomedica pg/ml	Roche pg/ml
mean	24.8	14.0	210.1	119,3
max	75.6	44.7	640.7	379,1
min	1.1	1.1	9.3	9,5