

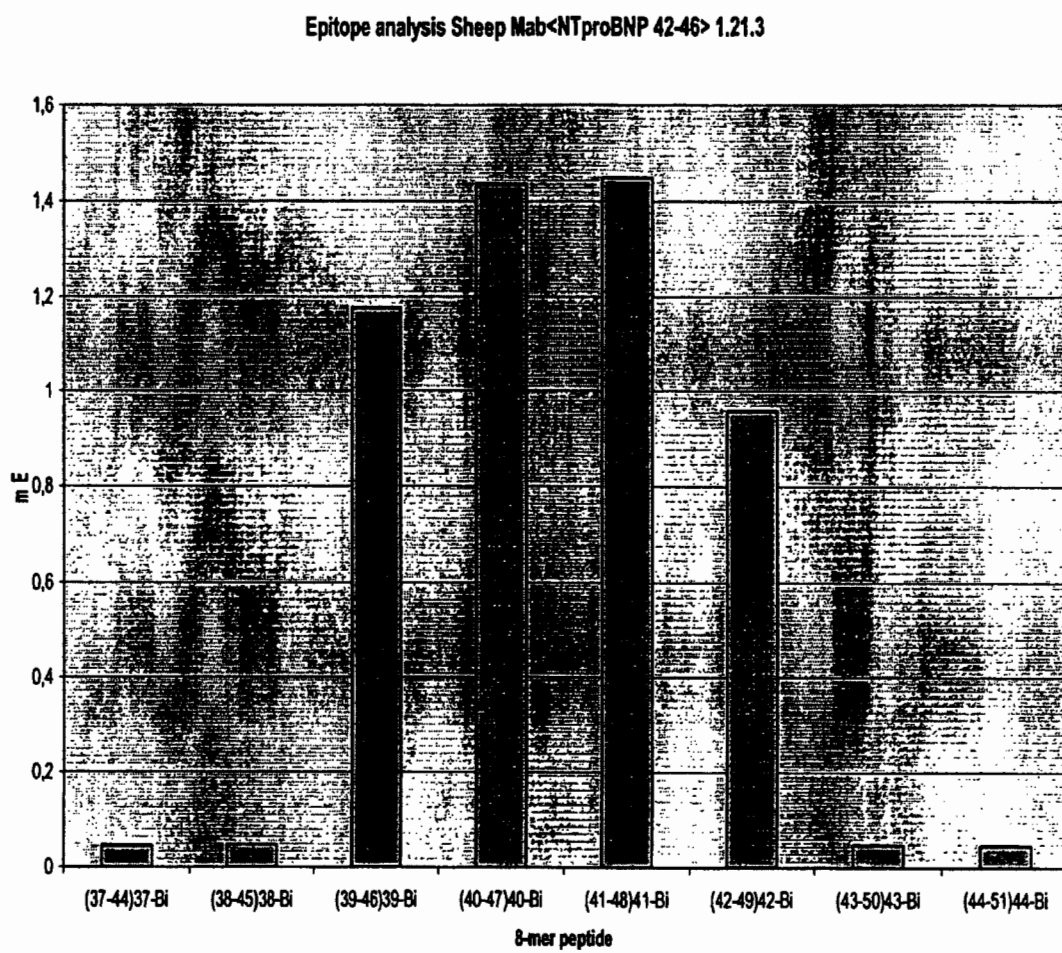
Fig. 1

Fig. 2a

Program for the determination of NT-proBNP in 20 patient samples with 8 different antibodies in Biacore written and to be executed with BIACORE 3000 Control Software Version. 4.1

DEFINE APROG Sandwich

PARAM %apo %anr %aco %bnr %bco %c1po %c1nr %c1co %c2po %c2nr %c2co %c3po %c3nr %c3co %c4po %c4nr %c4co

KEYWORD anr %anr
KEYWORD aco %aco
KEYWORD bnr %bnr
KEYWORD bco %bco
KEYWORD c1nr %c1nr
KEYWORD c1co %c1co
KEYWORD c2nr %c2nr
KEYWORD c2co %c2co
KEYWORD c3nr %c3nr
KEYWORD c3co %c3co
KEYWORD c4nr %c4nr
KEYWORD c4co %c4co

CAPTION Sandwich: AG: %anr %aco mit %bnr %bco, AB: %c1nr, %c2nr, %c3nr, %c4nr

FLOW 10 -f
FLOWPATH 1,2,3,4

DIPNEEDLE r2e1
* QUICKINJECT %apo 100 !calibrator/human serum
-0:10 RPOINT -b BL_start

FLOW 100
* QUICKINJECT r2f6 50 !HBS

FLOW 10 -f

FLOWPATH 1
DIPNEEDLE r2e2
* QUICKINJECT %c1po 30 !AB1/5
-0:10 RPOINT -b AG

FLOWPATH 2
DIPNEEDLE r2e3
* QUICKINJECT %c2po 30 !AB2/6
-0:10 RPOINT -b AB1

FLOWPATH 3
DIPNEEDLE r2e4
* QUICKINJECT %c3po 30 !AB3/7
-0:10 RPOINT -b AB2

FLOWPATH 4
DIPNEEDLE r2e5
* QUICKINJECT %c4po 30 !AB4/8
-0:10 RPOINT -b AB3

Fig. 2b

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FLOWPATH 1,2,3,4
FLOW 100

* QUICKINJECT r2f7 50 !HBS
-0:10 RPOINT -b AB4

FLOW 20

* QUICKINJECT r2e10 5 !HBSwash
* QUICKINJECT r2f3 10 !100 mM HCl
* QUICKINJECT r2f4 10 !100 mM phosphoric acid
* QUICKINJECT r2f5 10 !100 mM phosphoric acid
EXTRACLEAN
3:30 RPOINT BL_end !baseline after regen.cycle
END

DEFINE APROG Regen
CAPTION regeneration cycle
FLOW 20
FLOWPATH 1,2,3,4
* QUICKINJECT r2e10 5 !HBSwash
-0:10 RPOINT -b BL_start
* QUICKINJECT r2f3 10 !100 mM HCl
* QUICKINJECT r2f4 10 !100 mM phosphoric acid
* QUICKINJECT r2f5 10 !100 mM phosphoric acid
3:30 RPOINT BL_end !baseline after regen.cycle
END

DEFINE LOOP AG
LPARAM %apo %anr %aco %bnr %bco
TIMES 1
! %apo %anr %aco %bnr %bco
r2a1 NT-proBNP 40nM HoSer/CMD 20%/1mg/mL
r2a2 NT-proBNP 20nM HoSer/CMD 20%/1mg/mL
r2a3 NT-proBNP 10nM HoSer/CMD 20%/1mg/mL
r2a4 NT-proBNP 5nM HoSer/CMD 20%/1mg/mL
r2a5 NT-proBNP 2.5nM HoSer/CMD 20%/1mg/mL
r2a6 NT-proBNP 0nM HoSer/CMD 20%/1mg/mL
r2b1 HuSer1 1:5 HBS/CMD 1mg/mL
r2b2 HuSer2 1:5 HBS/CMD 1mg/mL
r2b3 HuSer3 1:5 HBS/CMD 1mg/mL
r2b4 HuSer4 1:5 HBS/CMD 1mg/mL
r2b5 HuSer5 1:5 HBS/CMD 1mg/mL
r2b6 HuSer6 1:5 HBS/CMD 1mg/mL
r2b7 HuSer7 1:5 HBS/CMD 1mg/mL
r2b8 HuSer8 1:5 HBS/CMD 1mg/mL
r2b9 HuSer9 1:5 HBS/CMD 1mg/mL
r2b10 HuSer10 1:5 HBS/CMD 1mg/mL
r2c1 HuSer11 1:5 HBS/CMD 1mg/mL
r2c2 HuSer12 1:5 HBS/CMD 1mg/mL
r2c3 HuSer13 1:5 HBS/CMD 1mg/mL
r2c4 HuSer14 1:5 HBS/CMD 1mg/mL
r2c5 HuSer15 1:5 HBS/CMD 1mg/mL

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Fig. 2c

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r2c6 HuSer16 1:5 HBS/CMD 1mg/mL
r2c7 HuSer17 1:5 HBS/CMD 1mg/mL
r2c8 HuSer18 1:5 HBS/CMD 1mg/mL
r2c9 HuSer19 1:5 HBS/CMD 1mg/mL
r2c10 HuSer20 1:5 HBS/CMD 1mg/mL
END

DEFINE LOOP AB
LPARAM %c1po %c1nr %c1co %c2po %c2nr %c2co %c3po %c3nr %c3co %c4po %c4nr %c4co
TIMES 1
  r1a1 AB1 500nM r1a2 AB2 500nM r1a3 AB3 500nM r1a4 AB4 500nM
  r1b1 AB5 500nM r1b2 AB6 500nM r1b3 AB7 500nM r1b4 AB8 500nM
END

MAIN
RACK 2 Thermo_a
RACK 1 Thermo_c

detection 1,2,3,4

LOOP AB ORDER
  APROG Regen
  unclog
LOOP AG ORDER
  APROG Sandwich %apo %anr %aco %bnr %bco %c1po %c1nr %c1co %c2po %c2nr %c2co
    %c3po %c3nr %c3co %c4po %c4nr %c4co
ENDLOOP
ENDLOOP

  APROG Regen
  APPEND continue
END

```

Fig. 3

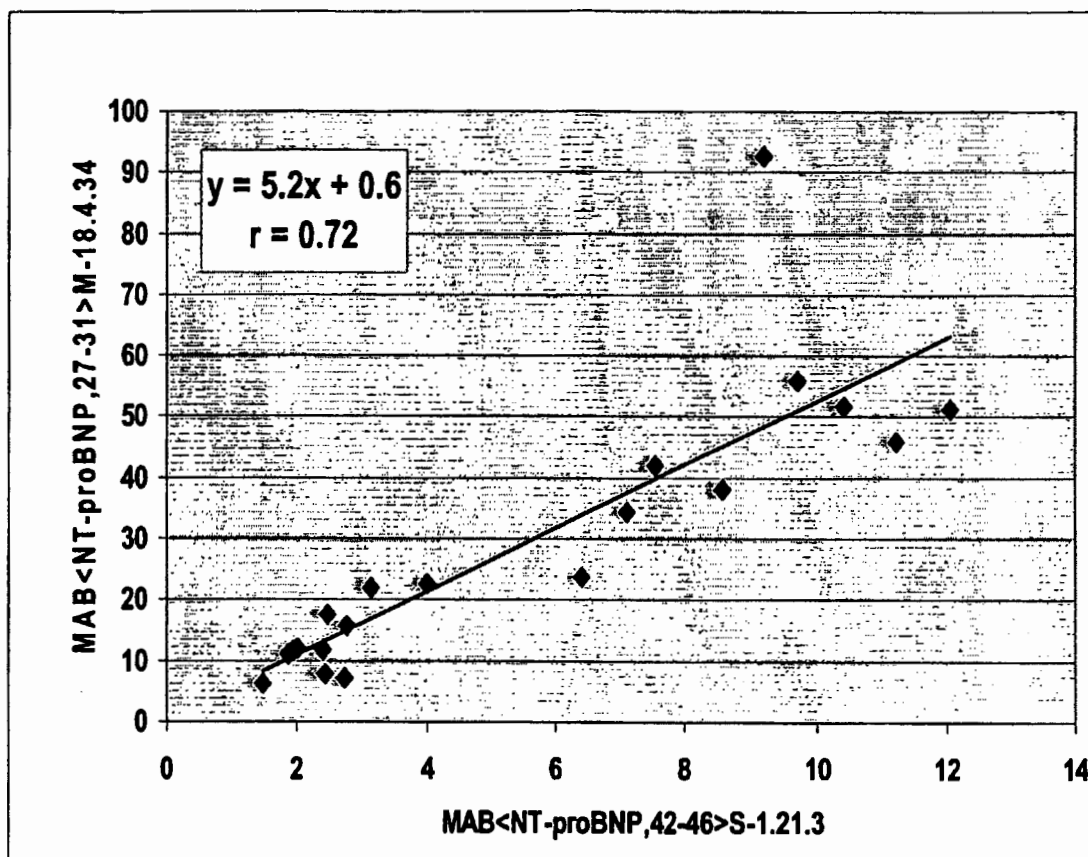


Fig. 4

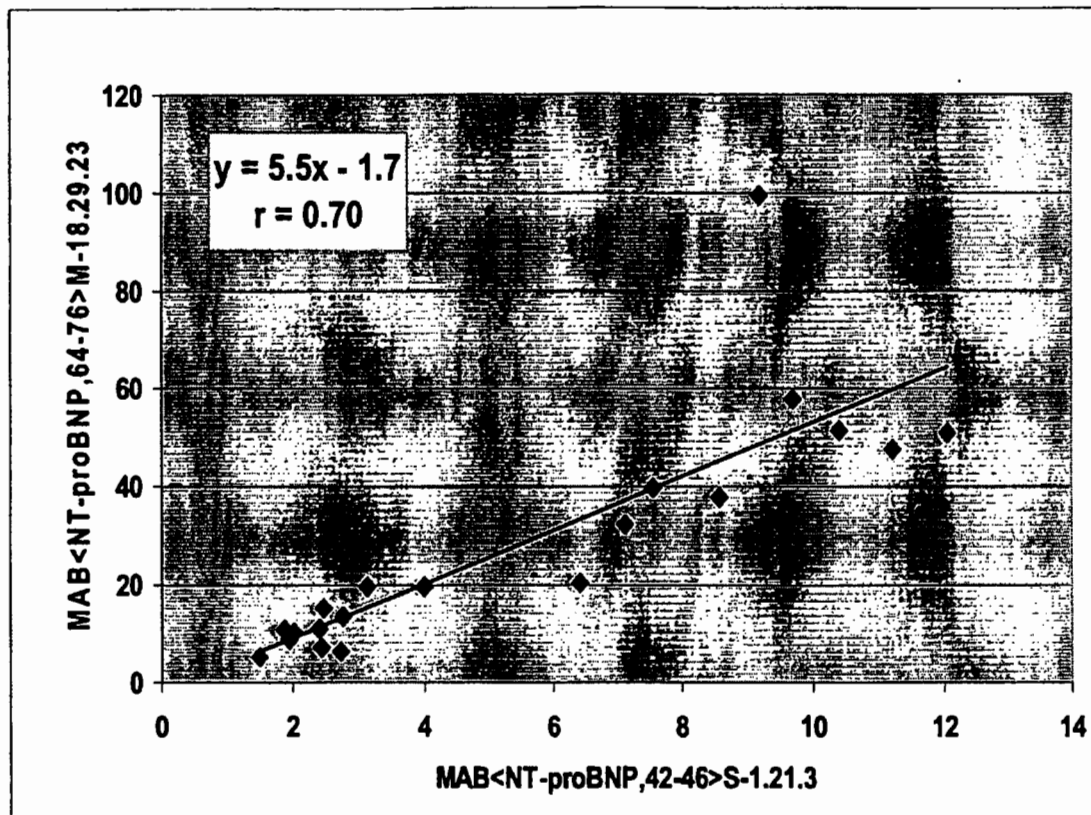


Fig. 5

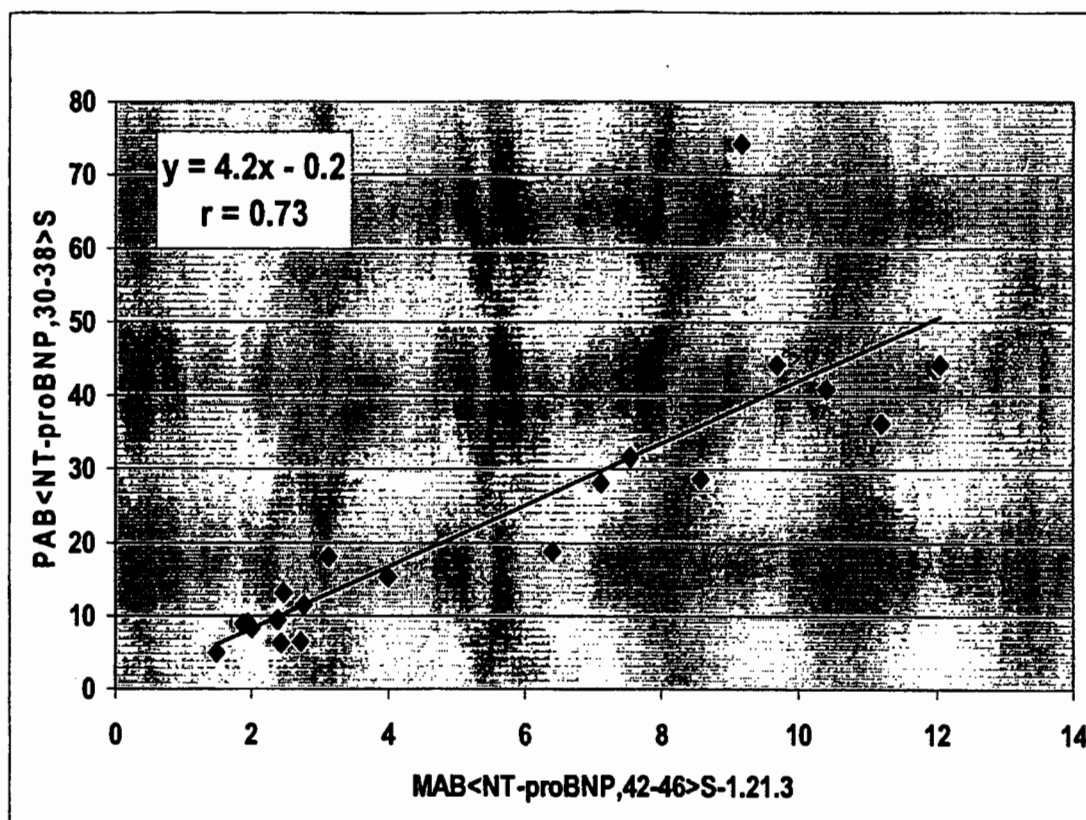


Fig. 6

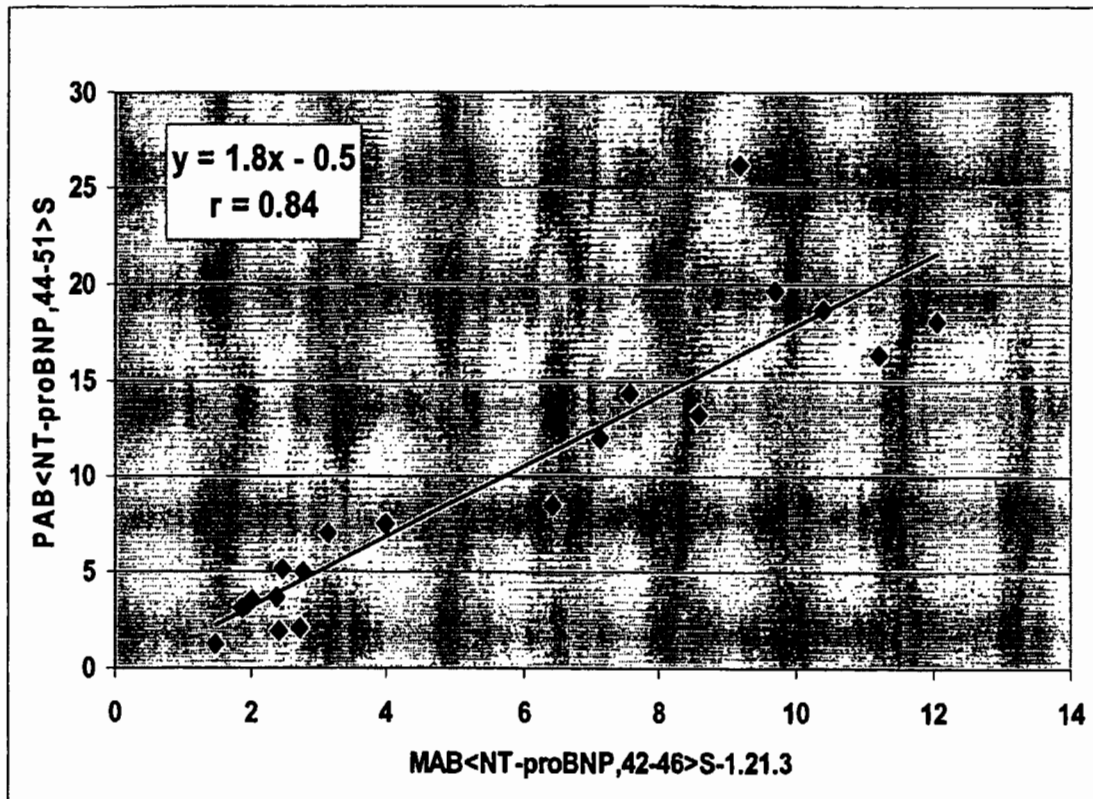


Fig. 7

