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Recombinant Proform of Brain Natriuretic Peptide (proBNP), Expressed in Eukaryotic Cells as a Stable Standard for BNP Immunoassay

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INTRODUCTION

Measurements of blood concentration of Brain Natriuretic Peptide (BNP) are useful for identifying patients with congestive heart failure (CHF). Commonly, synthetic BNP is used as a standard in BNP immunoassays. However it is known that synthetic BNP demonstrates low stability being reconstituted in plasma or buffer solutions. Because of low stability the use of synthetic BNP as a standard in BNP assay is limited. We have recently demonstrated that BNP-immunoreactivity in human blood mostly belongs to the proform of BNP – proBNP. It was also shown that endogenous proBNP circulates in blood as a glycoprotein. Relying on these data we suggested using recombinant proBNP as a calibrator for BNP immunoassays.
In current study we have compared stability of synthetic BNPs, recombinant proBNP, expressed in *E. coli* and recombinant glycosylated proBNP, expressed in CHO and human HEK 293 cells.

HyTest

Materials and Methods

Antigens: synthetic human BNPs were from Bachem AG (Switzerland) and from Peptide Institute (Japan). Recombinant glycosylated human proBNP, expressed in human HEK 293 and CHO cells and recombinant non-glycosylated proBNP, expressed in *E. coli* were from HyTest (Turku, Finland).

BNP immunoassay, utilizes MAb 50E1 (epitope 26-32) as capture and MAb 24C5 (epitope 11-22) as detection. In such assay the mixture of biotinylated capture MAb 50E1 and Eu^{3+}-labeled detection MAb 24C5 (50µL) and antigen, (50µL) are incubated for 30 min at room temperature in streptavidin-coated plate. The scheme of HyTest's BNP research immunoassay is presented on Fig. 1.

Stability studies: synthetic BNPs, recombinant non-glycosylated and glycosylated proBNP forms, reconstituted in pooled plasma from healthy volunteers were assessed by BNP research immunoassay 50E1 – 24C5. Antigens concentrations were: 6 ng/mL for recombinant proBNP expressed in CHO cells, 20 ng/mL for recombinant proBNP expressed in *E. coli* and recombinant proBNP expressed in HEK cells and synthetic BNPs. Samples were incubated at +4°C and at +25°C for different time periods up to 96 hours. 0.1% sodium azide was added to plasma as a preservative to prevent bacteria growth.

Results and Discussion

Sandwich immunofluorometric BNP assay

Sandwich immunoassay used in the study utilizes two monoclonal antibodies 50E1 and 24C5 (HyTest, Turku), specific to the regions 26-32 and 11-22 of BNP molecule, respectively. Such assay is able to detect not only BNP, but also proBNP with the same efficiency and can be used for the detection of whole BNP-immunoreactivity in the sample. Calibration curves for BNP 50E1 - 24C5 immunoassay are shown on Fig. 2.

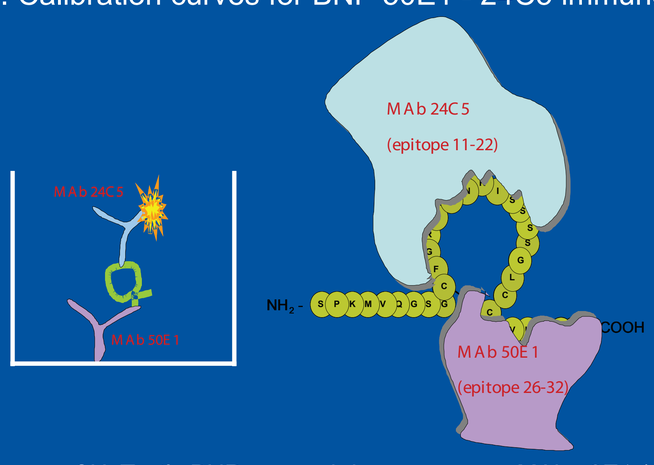

Figure 1:The scheme of HyTest's BNP research immunoassay. MAb 50E1 (capture) and 24C5 (detection) recognizes different epitopes of BNP molecule.

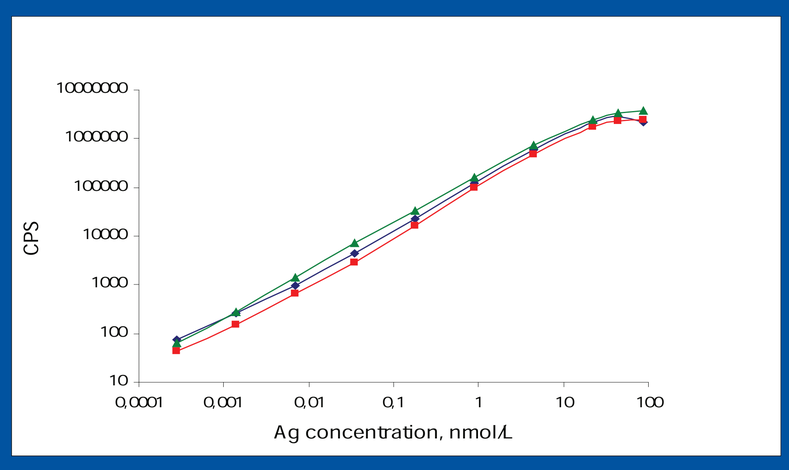

Figure 2: Calibration curves for 50E1-24C5 fluoroimmunoassay. Synthetic BNP (■), recombinant non-glycosylated proBNP (▲), recombinant glycosylated proBNP (■) were used as calibrators. The analytical sensitivity determined as 2 SD above the mean for a blank calibrator was 0.4 ng/L (synthetic BNP (Bachem) was used as a calibrator).

Stability studies

BNP is known as an unstable molecule and it rapidly loses its' immunological activity being reconstituted in human serum or plasma. Most likely this loss of activity is associated with proteolytic degradation of the peptide. We compared the stability of several peptides, displaying BNP-immunoreactivity to determine the most stable one. The stability was assessed by BNP 50E1 – 24C5 immunoassay. In Figure 3 we are presenting results of stability studies. Samples were incubated at +4°C (Fig. 3A) and at +25°C (Fig. 3B) for different time periods up to 96 hours. We have shown that synthetic BNPs demonstrated the worst stability among all tested forms. 3% and less of initial immunoreactivity was observed in the samples after 24 hours of incubation at +4°C and less than 0.5% after 24 hours of incubation at +25°C. After 96 hours of incubation at both temperatures no BNP-immunoreactivity was detected. Recombinant non-glycosylated proBNP (*E. coli*) demonstrated intermediate stability. More than 50% of initial proBNP-immunoreactivity was detected after 96 hours of incubation at +4°C, while less than 1% of initial proBNP immunoreactivity was detected after 96 hours of incubation at +25°C. Recombinant glycosylated forms of proBNP expressed in CHO and HEK cells demonstrated the best stability; with higher stability for the proBNP, expressed in HEK cells. More than 90% of initial immunoreactivity was detected for both proteins after 96 hours of incubation at +4°C, and 70% (CHO) and 82% (HEK) of initial immunoreactivity after 96 hours of incubation at +25°C.

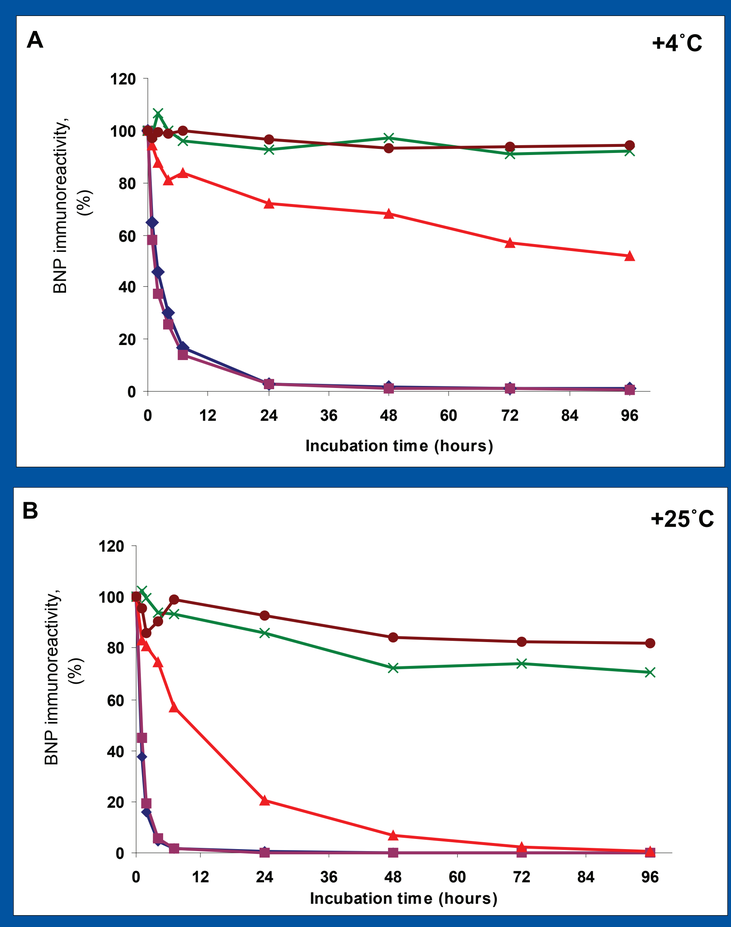

Figure 3: Stability of BNP and proBNP forms assessed by BNP immunoassay 50E1-24C5. BNP, Bachem (♦), BNP, Peptide Institute (■), recombinant proBNP, expressed in *E. coli* (▲), recombinant proBNP, expressed in CHO cells (×), recombinant proBNP, expressed in HEK cells (●). Antigens were reconstituted in normal human plasma and incubated +4°C (A) and at +25°C (B) for different time periods.

Conclusions

Synthetic BNPs demonstrated the worst stability among all tested molecules, displaying BNP-immunoreactivity. Recombinant proBNPs are more stable. Glycosylated proBNPs, expressed in eukaryotic cell lines, have the highest stability. Taking into account results of stability studies and the prevalence of proBNP in human blood, we are suggesting here to use proBNP, expressed in eukaryotic cells, as a stable standard or calibrator in BNP immunoassays.