



Antibodies for New Type of BNP immunoassay – “Single Epitope Sandwich” (SES) assay



Brain natriuretic peptide (BNP) is an acknowledged marker of heart failure (HF) that is widely used in clinical practice for HF diagnosis and patient management.

BNP is known as an unstable molecule (1, 2). Several recent studies have revealed that BNP is presented by multiple forms in

HF patients' plasma, truncated from both N- and C-termini and only a small portion of BNP circulates as a full-size BNP32 molecule (3). The majority of commercially available BNP assays are designed as sandwich-type immunoassays utilizing two MAbs specific to distantly located epitopes. At least one of these two antibodies is specific to the ring structure, while the other one is usually specific to the C-terminus of the BNP molecule. Recent data regarding BNP instability in circulation suggests that immunoassays utilizing at least one MAb specific to the terminal epitope could underestimate the real BNP content in the blood sample.

HyTest specialists have developed antibodies for a brand new type of BNP immunoassay – the “**Single Epitope Sandwich**” immunoassay (**SES assay**) - which differs from all commercially available “conventional”-type sandwich BNP assays (4). In the SES assay the capture antibody (MAb 24C5, epitope 11-17), which is specific to the relatively stable ring part of BNP molecule recognizes antigen. The detection antibody is specific only to the complex of the capture antibody with the BNP (or proBNP) and does not recognize

these two molecules, (capture antibody and BNP) separately. Therefore only a single epitope of BNP molecule is needed for this novel type of sandwich BNP immunoassay. This feature provides additional advantages to the SES assay over conventional BNP assays in terms of a higher apparent stability of BNP antigen in the sample or bloodstream.

Sensitivity of the SES assay. The prototype assay, designed by HyTest specialists to evaluate antibodies and the principle of the single epitope sandwich is a one-step assay utilizing biotinylated capture MAb 24C5 and detection MAb Ab-BNP2 labeled with stable Eu^{3+} chelate. Both MAbs and antigen are simultaneously incubated in streptavidin-coated plates and the assay time is 35 min. The limit of detection of the SES assay is 0.4 pg/ml (human synthetic BNP, Peptide Institute, Japan). This is the highest sensitivity described in literature for all commercial and experimental BNP assays. A detailed description of the prototype SES BNP assay has recently been published (4).

Interaction with BNP and proBNP forms. According to the recent studies, the major portion of BNP immunoreactivity in the patient's blood is not presented by BNP, but in fact is presented by proBNP (5, 6). To be precise in measurements of BNP immunoreactivity in the sample, assay antibodies should recognize BNP and proBNP with the same efficiency.

The SES assay recognizes three forms displaying BNP immunoreactivity – BNP, non-glycosylated proBNP and glycosylated proBNP – with the same efficiency (Fig. 1). When the SES BNP assay was tested with plasma samples of HF patients, it was shown to be suitable for precise quantification of circulating BNP and proBNP molecules.

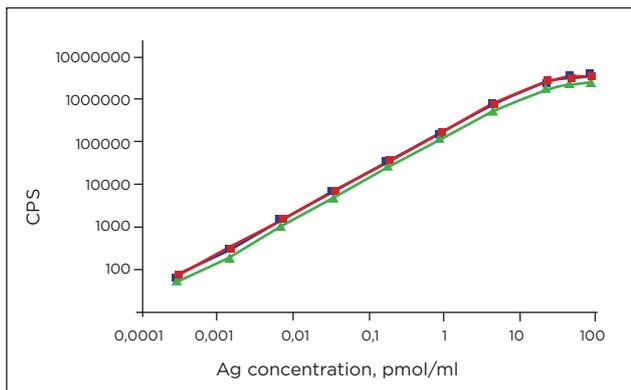


Figure 1. Recognition of different antigen forms displaying BNP-immunoreactivity by SES assay. Calibration curves for three different antigens: ■- synthetic BNP, ■- recombinant proBNP (glycosylated, expressed in mammalian cell line; HyTest Cat.# 8GOB2), ▲- recombinant proBNP (non glycosylated, expressed in *E. coli*).

Antigen stability studies. As it was mentioned above, BNP is known as a very unstable molecule that is easily cleaved by endogenous proteases in human blood. However, in SES assay, in which MAbs need the single relatively stable central epitope for sandwich formation, BNP displays significantly higher stability, than when measured in conventional sandwich assays, comprising antibodies with distant epitopes. In Fig. 2 results of stability studies of synthetic BNP and endogenous antigen from plasma of HF patients are presented. Stability was assessed by SES assay and conventional type assay prototype utilizing MAbs with distant epitopes - MAb 50E1 as capture and MAb 24C5 as detection. Samples were

incubated at room temperature for different time periods lasting up to 24 hours. Compared to the conventional sandwich assay, the apparent stability of the synthetic antigen is significantly higher when measured by SES assay. While approximately 95% of BNP immunoreactivity was observed with SES assay after 24 hours of incubation, only 62% of initial BNP immunoreactivity was detected when the conventional sandwich immunoassay was used. Furthermore, the apparent stability of the endogenous peptide was also higher when BNP immunoreactivity in individual HF plasma sample incubated for different time periods was measured by the SES assay.

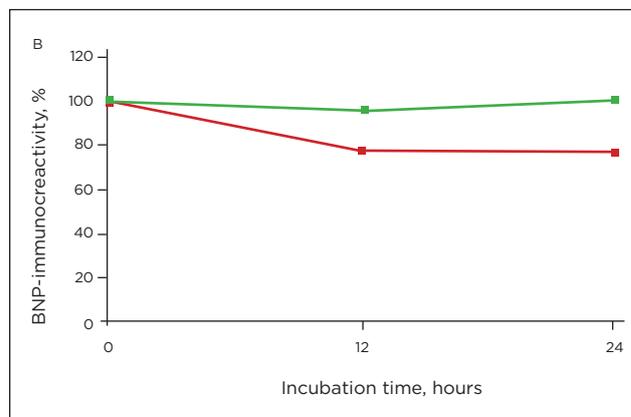
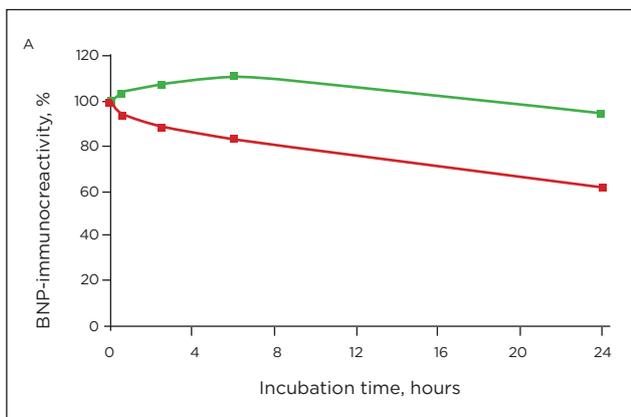


Figure 2. Stability studies of BNP.

(A) Synthetic BNP (Peptide Institute, Japan) reconstituted in individual normal human EDTA-plasma and **(B)** Individual HF patient EDTA-plasma were incubated at RT (24°C) for different time periods. BNP immunoreactivity was tested in the SES assay (■-) and conventional type BNP assay utilizing two MAbs 50E1 and 24C5 specific to the distant epitopes (■-).

BNP measurements in HF plasma. BNP immunoreactivity measurements (BNP and proBNP) in individual EDTA-plasma samples of HF patients performed by two types of BNP assays also revealed the superiority of the single epitope principle over the conventional one. BNP concentration in 94 HF plasma samples was quantified by SES assay prototype and by commercially available conventional type Siemens ADVIA Centaur BNP immunoassay. The Siemens assay utilizes one MAb specific to the C-terminus (epitope 27-32) and another MAb specific to the ring

structure of BNP molecule (epitope 14-21) (7). Both assays were calibrated with recombinant proBNP, expressed in *E. coli*. In all plasma samples, when compared with the results obtained by Siemens BNP immunoassay, SES assay detected significantly more BNP; from 1.2 to 7.2-fold; 2.1 ± 0.9 (mean \pm SD). BNP concentration in seven plasma samples (7.4% of a total of 94 samples) when measured by SES assay was from 3 to 7.2-fold higher than that measured by Siemens assay. Results of the BNP measurements in 94 HF patients plasma are presented in Fig. 3.

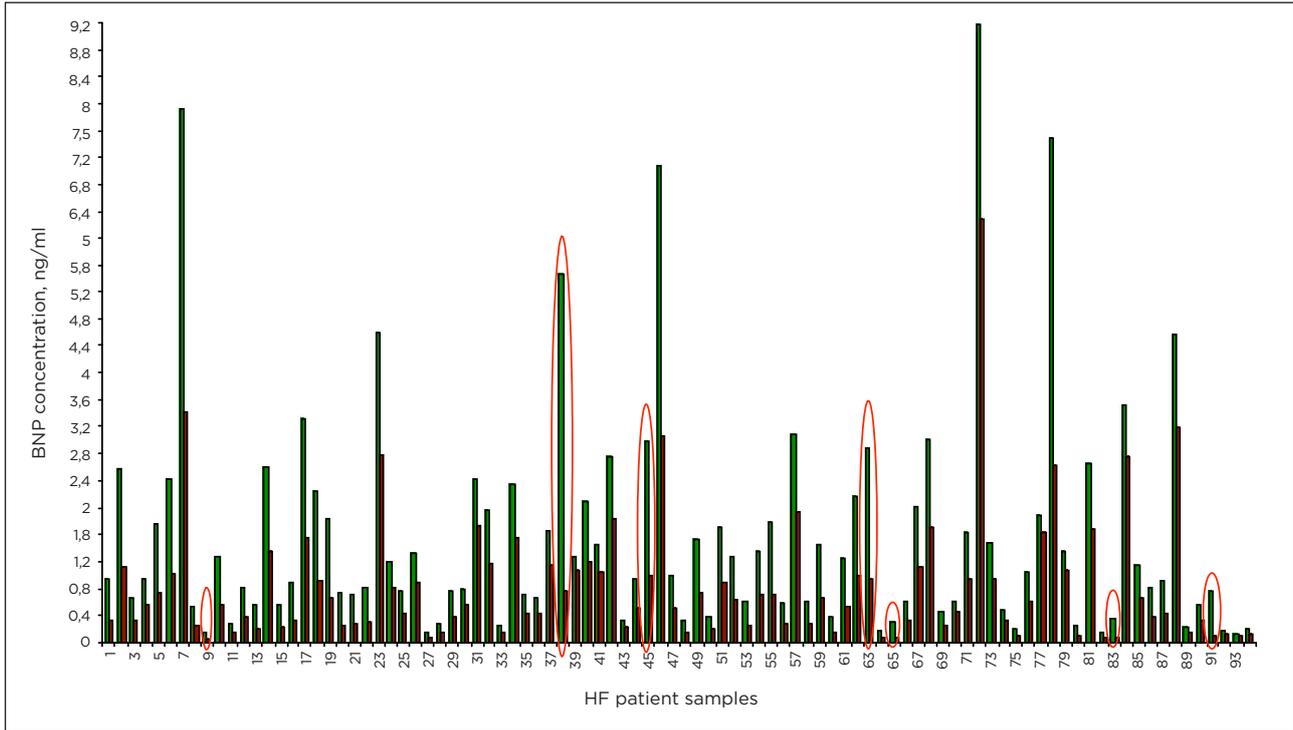


Figure 3. BNP measurements in 94 HF patients’ plasma samples. BNP concentration was measured by the SES (green bars) and Siemens (brown bars) BNP assays. Cases when the concentrations measured by SES assay were 3 to 7.2-fold higher than those measured by Siemens assay are marked by red ovals.

As can be seen from Fig. 3 Siemens assay underestimates circulating BNP concentrations in HF patients. This observation can at least be explained by the fact that one of the MAbs, utilized in the Siemens assay, is specific to the epitope 27-32 and cannot recognize BNP forms truncated from the C-terminus. The HyTest SES assay, being significantly less sensitive to the proteolytic degradation of the antigen, is capable of detecting all forms displaying BNP-immunoreactivity: intact and terminus-truncated antigens. The SES assay appears to be a preferable assay for the absolute BNP quantification in human blood.

The true BNP values are required to make a correct diagnosis for patients admitted to emergency department (ED) with symptoms of HF. Specific “rule out” (BNP<100 pg/ml) and “rule in” (BNP>400 pg/ml) values are currently used by cardiologists to make the most accurate diagnosis in ED (19). Fig. 4 represents the difference in BNP content for four selected patients measured by SES and Siemens assays. Being measured by the Siemens BNP assay these patients (especially patient #4) could be misclassified (uncertainty zone: concentration range from 100 pg/ml to 400 pg/ml) and could therefore be mistakenly diagnosed. When measured by the SES assay, the same patients undoubtedly belong to the “rule in” zone. This example confirms the idea that the SES assay approach is a preferable for the precise BNP quantification.

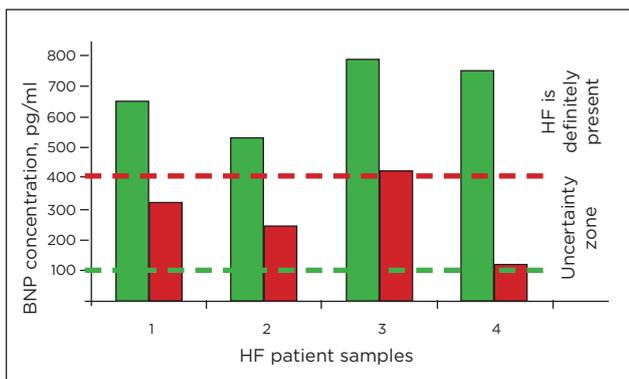


Figure 4. BNP measurements in plasma of four selected HF patients by two BNP assays. BNP concentration was measured by the SES (green bars) and Siemens (red bars) assays. When measured by the Siemens assay these patients may have unconfirmed HF (BNP concentrations in the range of 100-400 pg/ml), whereas measured by the SES assay - confirmed HF (more than 400 pg/ml). The lower limit of the uncertainty zone or unconfirmed HF (100 pg/ml) is marked as a green dash line; the lower limit of the zone where HF is definitely present (400 pg/ml) and is marked as red dash line.

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IMMUNOASSAY FOR QUANTIFICATION OF AN UNSTABLE ANTIGEN SELECTED FROM BNP AND PROBNP

Ordering information**MONOCLONAL ANTIBODIES**

| Product name | Cat. # | MAb | Subclass | Remarks |
|----------------------------------|--------|---------|----------|---|
| BNP | 4BNP2 | 25C5 | IgG1 | EIA, WB, a.a.r. 11-17 |
| Immune complex (24C5-BNP/proBNP) | 4BFab5 | Ab-BNP2 | IgG2a | EIA (only as pair with MAb 24C5, Cat.# 4BNP2) |
| Immune complex (24C5-BNP/proBNP) | 4BFab5 | Ab-BNP4 | IgG2a | EIA (only as pair with MAb 24C5, Cat.# 4BNP2) |

ANTIGENS

| Product name | Cat. # | Purity | Source |
|-----------------------------------|--------|--------|-------------|
| ProBNP, recombinant | 8PRO9 | >95% | Recombinant |
| ProBNP, glycosylated, recombinant | 8GOB2 | >95% | Recombinant |

References

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