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NT-proBNP Antibodies with Distinct Binding Epitopes Applicable to Multiple Diagnostic Platforms

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NT-proBNP: a marker of acute heart failure

Timely and accurate diagnosis of acute heart failure (HF) is crucial for the initiation of adequate treatment. N-terminal B-type natriuretic peptide (NT-proBNP; Figure 1), released in response to myocardial injury, is a gold-standard diagnostic and prognostic biomarker for HF1, and an independent predictor of stroke2. NT-proBNP assessment is recommended by major cardiology societies for guiding the evaluation, categorization, and management of patients with HF worldwide3,4.

In vitro immunoassays are valuable tools in the rapid detection of elevated NT-proBNP concentrations (>125 pg/mL) in serum5. Determining the NT-proBNP monoclonal antibody (mAbs) pairs with optimal sensitivity in each assay platform is crucial for ensuring the highest possible clinical performance of different diagnostic NT-proBNP tests.

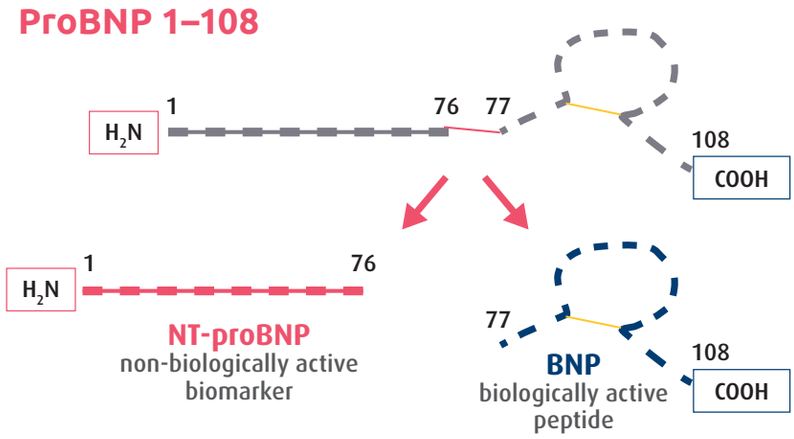

FIGURE 1. Synthesis of NT-proBNP from proBNP. The precursor protein proBNP 1–108 is proteolytically cleaved into NT-proBNP and BNP1.

Materials & Methods

We have developed seven mouse anti-human NT-proBNP mAbs—1306 (#100521), 1307 (#100719), 1308 (#100712), 1309 (#100710), 1310 (#100718), 1311 (#100716), and 1312 (#100717; all Medix Biochemica)—that bind to specific linear epitopes of NT-proBNP (Figure 2).

NT-proBNP capture and detection mAb pairs, with distinct antigen-binding epitopes, were selected for sensitivity assessment in three assay platforms:

1. Europium-based fluorescence immunoassay (FIA)
2. Acridinium ester-mediated chemiluminescence immunoassay (CLIA)
3. Colloidal gold lateral flow chromatography (LF).

Purified human NT-proBNP (#610090, Medix Biochemica) was used as an antigen. Selected antibody pairs were also assessed for inter-assay agreement between CLIA and a reference NT-proBNP assay from Roche on 18 clinical sera with varying NT-proBNP concentrations.

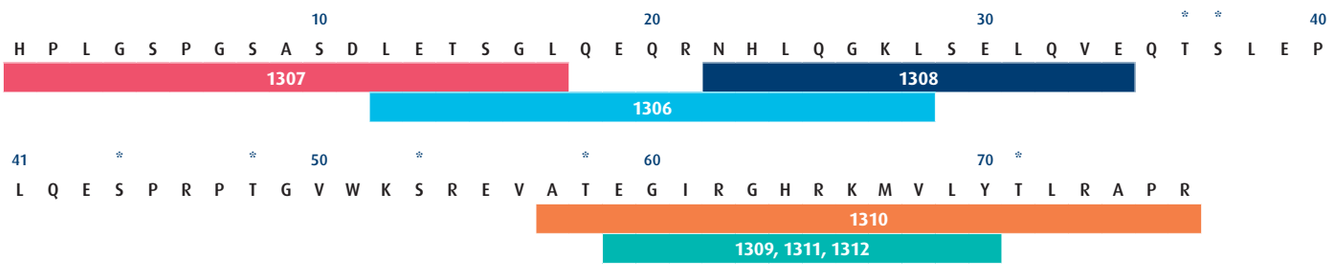

FIGURE 2. Binding epitopes of NT-proBNP mAbs. NT-proBNP is the N-terminal part of proBNP consisting of amino acids 1–76 (UniProt P16860). * potential glycosylation sites.

Assay-dependent NT-proBNP mAb pairing

Each assay was shown to have its optimal NT-proBNP mAb pair, with the linear detection range reaching below 100 pg/mL for the most sensitive pairs. In FIA the optimal pair was 1306+1312, while in CLIA the optimal pairs were 1308+1309 and 1308+1311 and in LF pair 1308+1311 (Figure 3). Overall, mAb 1309 was shown to be an excellent detection mAb that paired well with capture mAbs 1306, 1307 or 1308, depending on the assay platform (Figure 3).

Several NT-proBNP mAb pairs were shown to perform well across different platforms (Table 1). Antibody pairs 1308+1309 and 1306+1309 were chosen for further assessment on clinical samples in CLIA, which is one of the most frequently utilized NT-proBNP immunoassays.

NT-proBNP mAb pairs 1306+1309 and 1308+1309 exhibited excellent inter-assay correlation in CLIA and a gold-standard reference assay from Roche in NT-proBNP detection on a panel of clinical serum samples (r=0.997 and r=0.999, respectively; Figure 4).

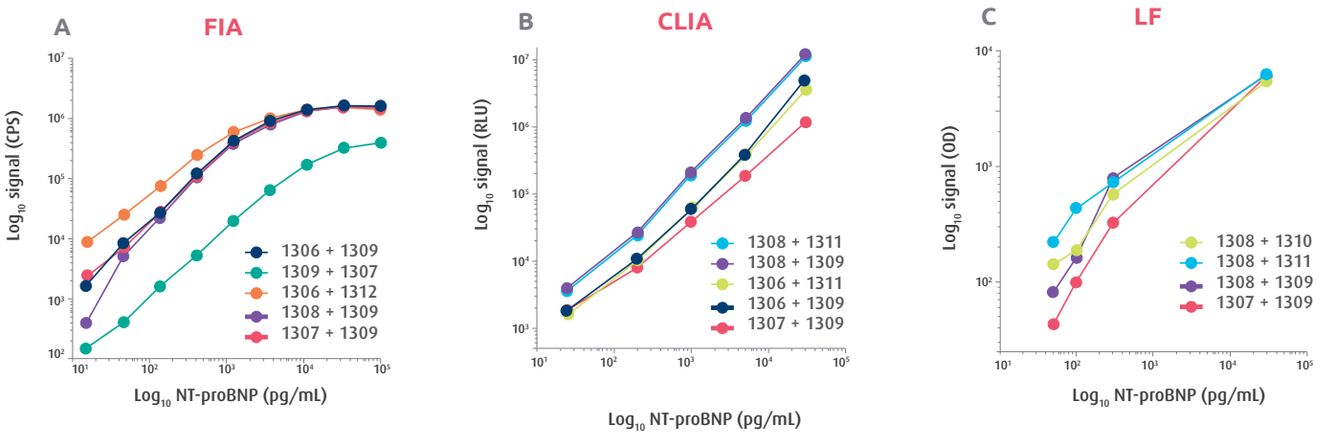

FIGURE 3. Standard curves for NT-proBNP detection by mAb pairs in A) FIA, B) CLIA, and C) LF. In the legend, the capture mAb is marked first and detection mAb second. CPS, counts per second; RLU, relative light units; OD, optical density.

TABLE 1. NT-proBNP mAb pairing properties by FIA, CLIA and LF. + mAbs work as pairs; - mAbs don't work as pairs. The optimal mAb pairs for each assay platform are highlighted: FIA, blue; CLIA, striped, and LF, red.

Capture \ Detection	1306	1307	1308	1309	1310	1311	1312
1306	–	+	–	+	+	+	+
1307	–	–	+	+	+	+	+
1308	–	+	–	+	+	+	+
1309	+	+	+	–	–	–	–
1310	+	+	+	–	–	–	–
1311	+	+	+	–	–	–	–
1312	+	+	+	–	–	–	–

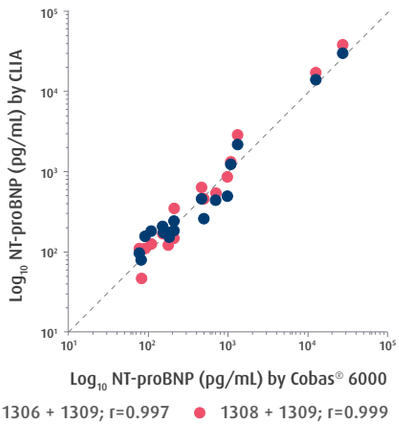

FIGURE 4. CLIA results with NT-proBNP mAb pairs 1306+1309 or 1308+1309 demonstrated an excellent correlation with Roche NT-proBNP assay on clinical serum samples. In the legend, capture mAb is marked first and detection mAb second. r, correlation coefficient.

Conclusions

We have demonstrated the applicability of several well-performing NT-proBNP mAb pairs for each of the three assay platforms assessed in this study. Furthermore, the mAb pairs that were also tested in a panel of clinical serum samples with varying NT-proBNP levels exhibited an excellent inter-assay correlation between CLIA and a gold-standard test from Roche.

The sensitivity of an NT-proBNP mAb pair is assay-dependent, directly influencing the assay outcome. Therefore, pre-determining an optimal mAb pair for each immunoassay is highly recommended for ensuring sensitive NT-proBNP detection and accurate HF diagnostics in a clinical setting.

Acknowledgements

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References

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