T218

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

William Sun[1], Sari Tiitinen[2], Laura-Leena Kiiskinen[2]

[1]Medix Biochemica China, Room 402, Building 21, No. 588 Tianxiong Rd. Zhoupu, Pudong, Shanghai 201321, China [2]Medix Biochemica Oy, Klovinpellontie 3, FI-02180 Espoo, Finland

Medix Biochemica

23rd IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine
May 19-23, 2019
Barcelona, Spain

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 HF[1], and an independent predictor of stroke[2]. NT-proBNP assessment is recommended by major cardiology societies for guiding the evaluation, categorization, and management of patients with HF worldwide[3,4].

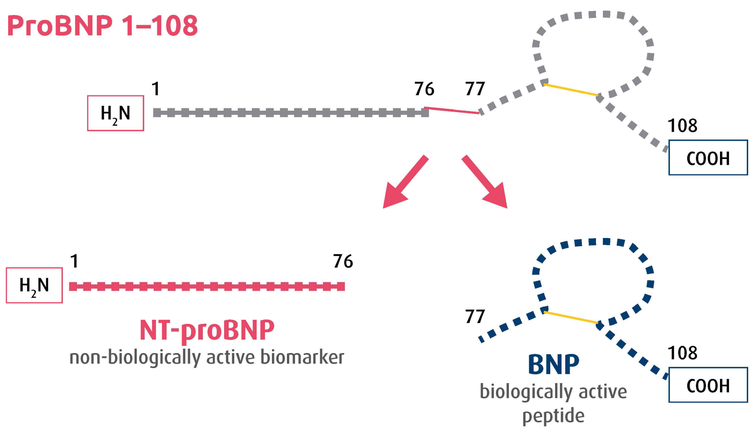

Figure 1. *Synthesis of NT-proBNP from proBNP. The precursor protein proBNP 1–108 is proteolytically cleaved into NTproBNP and BNP1.*

In vitro immunoassays are valuable tools in the rapid detection of elevated NT-proBNP concentrations (>125 pg/mL) in serum[5]. 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.

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).

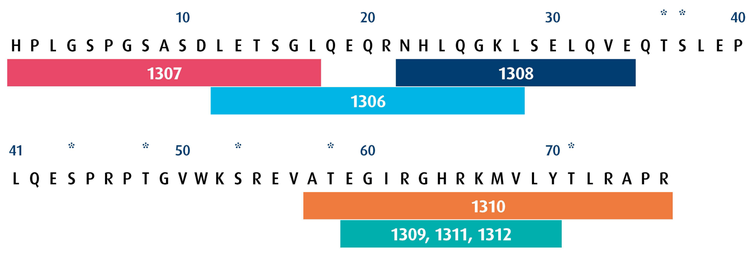

Figure 2. *Binding epitopes of NT-proBNP mAbs. NT-proBNP is the N-terminal part of proBNP consisting of amino acids 1–76 (UniProt P16860). * potential glycosylation sites.*

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.

Assay-dependent NT-proBNP mAb pairing

Each assay was shown to have its optimal NTproBNP 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).

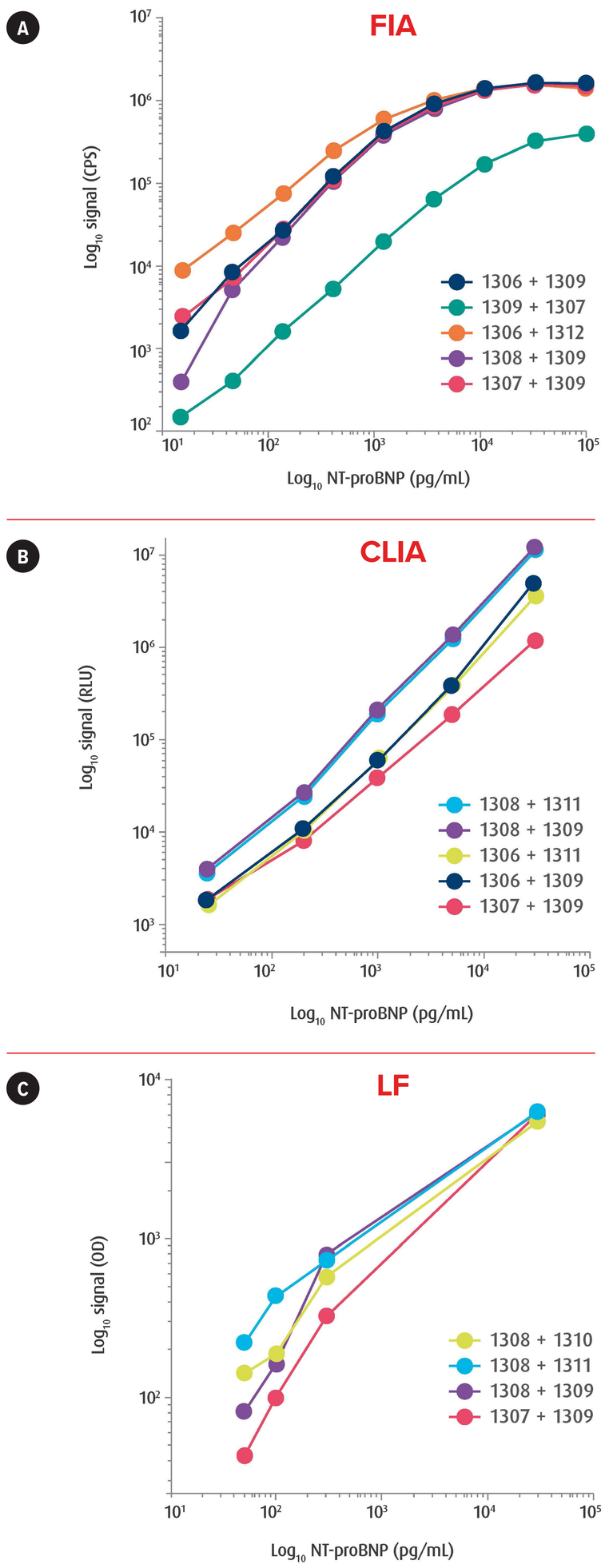

Figure 3. *Standard curves for NT-proBNP detection by mAb pairs in **A)** FIA, **B)** CLIA, and **C)** LF. In the legend, the capture mAb is marked first and detection mAb second. CPS, counts per second; RLU, relative light units; OD, optical density.*

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.

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

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.***

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).

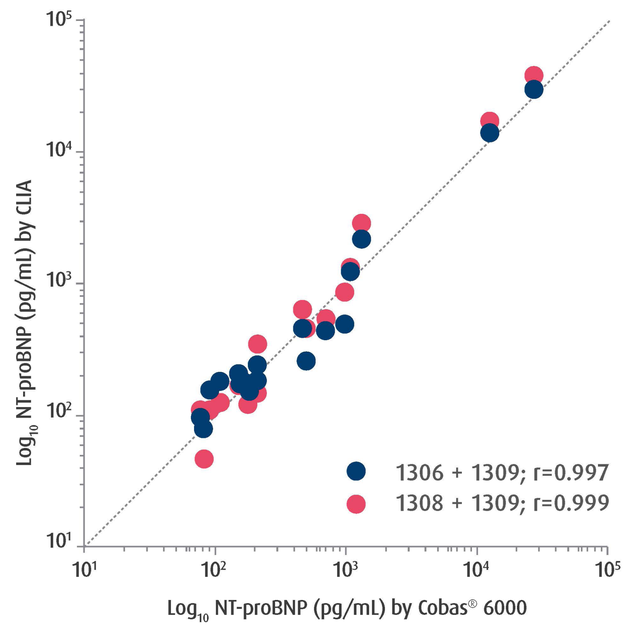

Figure 4. *CLIA results with NT-proBNP mAb pairs 1306+1309 or 1308+1309 demonstrated an excellent correlation with Roche NT-proBNP assay on clinical serum samples. In the legend, capture mAb is marked first and detection mAb second. r, correlation coefficient.*

Conclusion

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

We wish to thank our Laboratory Technicians for their excellent technical assistance in this study.

References

1. McKie et al. NT-proBNP: The gold standard biomarker in heart failure. J Am Coll Cardiol. 2016;68(22):2437–2439.

2. Castelnuovo et al. NT-proBNP (N-terminal pro-B-type natriuretic peptide) and the risk of stroke. Stroke. 2019;50(3):610–617.

3. Suzuki et al. Editor's Choice-Biomarkers of acute cardiovascular and pulmonary diseases. Eur Heart J Acute Cardiovasc Care. 2016;5:416–433.

4. Januzzi & Richards. Natriuretic peptide-guided heart failure therapy after the GUIDE-IT study. Circulation. 2018;137(20):2101–2103.

5. Ponikowski et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J. 2016; 37:2129–2200.

Medix Biochemica
Klovinpellontie 3, FI-02180 Espoo, Finland
medix@medixbiochemica.com • www.medixbiochemica.com