Novel High Affinity Monoclonal Antibodies for Specific Detection of Neurofilament Light Protein in Serum

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Introduction

Neurofilaments are very important protein components of axons, and they maintain the function of nervous system. Cerebrospinal fluid and serum neurofilament levels elevate due to damages in nervous system making neurofilaments promising biomarkers for neural diseases and injuries, such as Alzheimer's disease, traumatic brain injury and Multiple sclerosis. Blood matrix allows for repeated and less invasive monitoring of patients.[1-3]

Based on their size, neurofilaments are divided in groups: Neurofilament light (NfL), neurofilament medium (NfM), neurofilament heavy (NfH), α-internexin, and peripherin. Of these, NfL is considered to be the most promising biomarker since it is the most abundant form of neurofilaments.[1-3]

We have developed three novel mouse monoclonal antibodies (mAbs) against human NfL. In this study, binding properties of these antibodies were evaluated in fluorescence-based immunoassays (FIA).

Materials & Methods

Three anti-human NfL mAbs, designated as 12601 (#100984), 12603 (#100985) and 12604 (#100986), were assessed for their specificities and sensitivities. The antibodies were compared against commercially available reference antibodies in each experiment.

The cross-reactivities were studied in direct coating FIA with recombinant human NfL (#LA666), native bovine NfL, NfM, NfH, recombinant human Neuron-specific enolase (NSE, #610150) and native human S100 calcium binding protein B (S100B). Europium-labeled anti-mouse IgG was used for detection.

Selected antibody pairs were tested in sandwich FIA with NfL spiked normal serum and buffer to determine sensitivities, linear measuring ranges and the matrix effect. Biotin-conjugated antibodies and europium-labeled streptavidin were used for detection.

Results

In direct coating FIA, all three antibodies showed specificity for both recombinant human NfL and native bovine NfL (Fig. 1A). None of the antibodies cross-reacted with NfM, NfH, NSE or S100B (Fig. 1B and 1C).

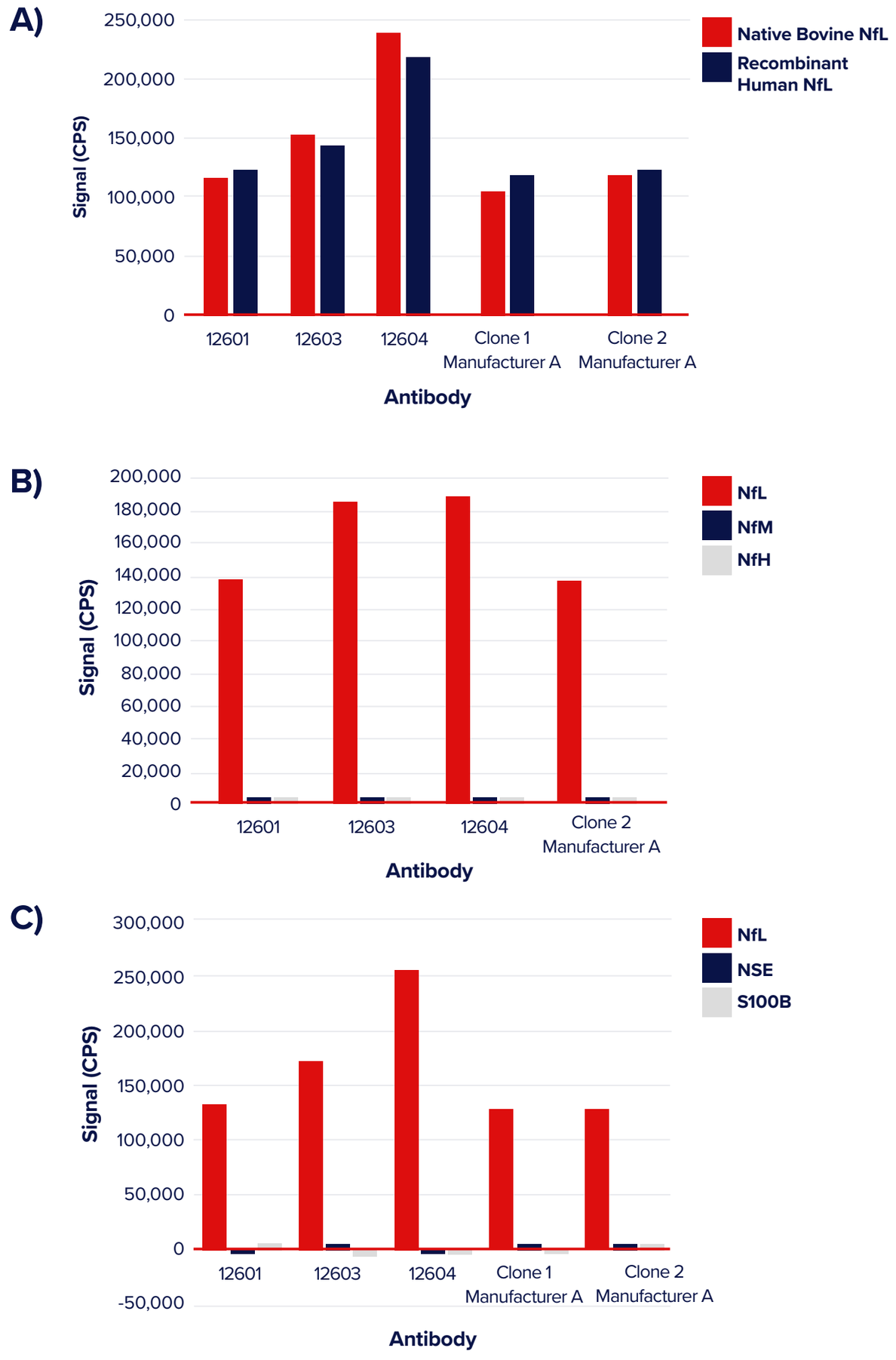

Fig. 1. Cross-reactions of the NfL antibodies to a) native bovine NfL, b) NfM and NfH and c) NSE and S100B in direct coating FIA.

In sandwich FIA, the widest linear measuring range of 10 to 40 960 pg/mL of recombinant human NfL spiked in normal serum was achieved with antibody pair 12603 and 12604. In contrast, the commercial reference antibody pair achieved a linear measuring range of 2 560 to 40 960 pg/mL (Fig. 2A). With NfL spiked buffer even 6 pg/mL could be distinguished from the background with each tested pair. However, Pearson's R for pairs 12603 + 12601 and 12603 + 12604 was 1.00 and 0.99, respectively, but only 0.18 for pair from Manufacturer A (Fig. 2B). In practice, matrix effect caused by serum was negligible for Medix Biochemica NfL mAbs.

Conclusions

High sensitivity immunoassays for NfL in blood samples allows for repeated and minimally invasive diagnosing and monitoring of patients suffering from neurological diseases. This study demonstrates that the novel NfL mAbs recognize both recombinant and native NfL in spiked serum samples. Comparison of Medix Biochemica mAbs to a commercially available pair indicated that higher sensitivity and wider detection range is achieved by using mAbs developed by Medix Biochemica.

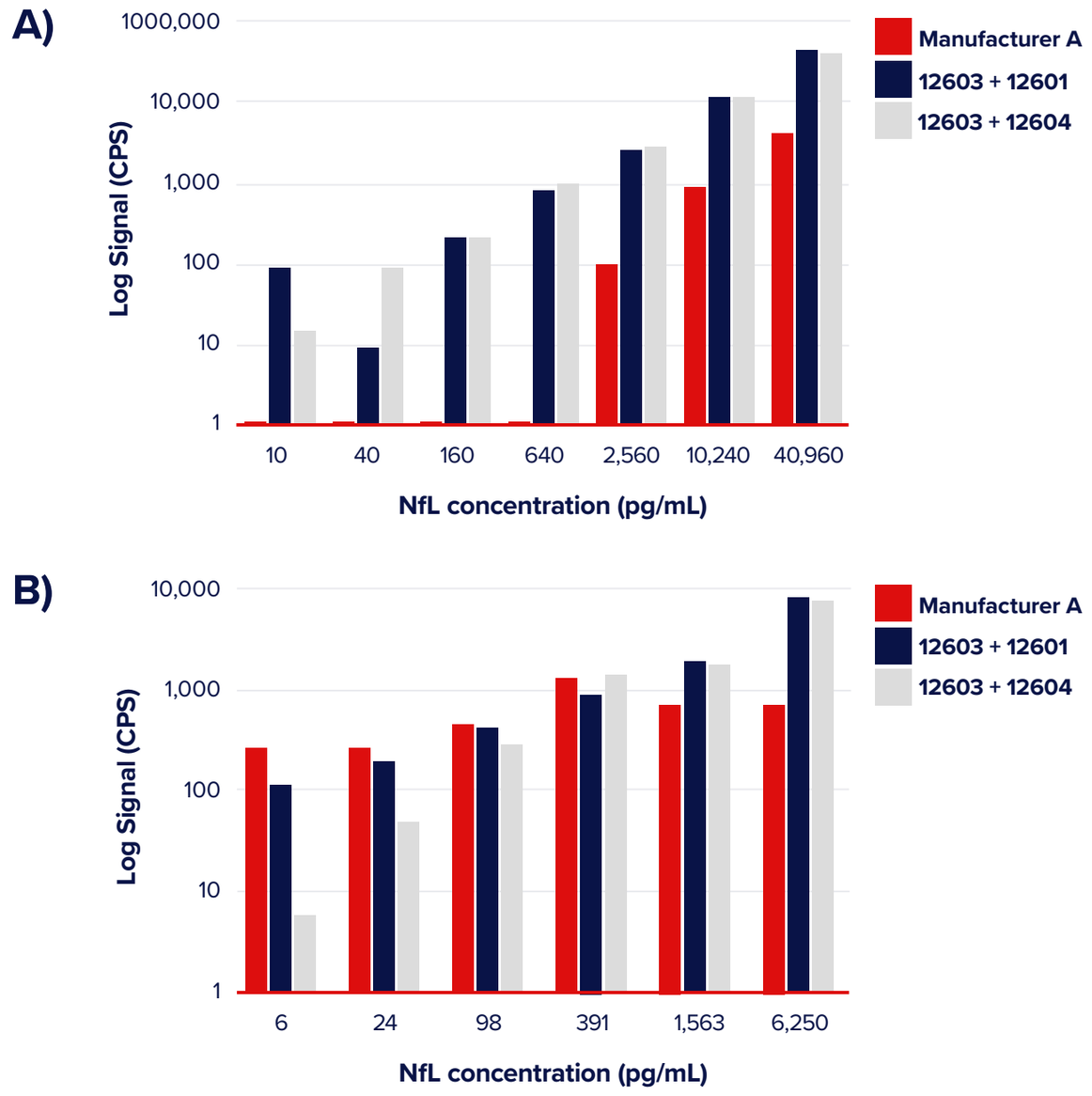

Fig. 2. Sensitivity and linear measuring ranges of the antibody pairs were tested in sandwich FIA with recombinant human NfL spiked in a) pooled normal serum and b) assay buffer.

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