Procollagen type I N-propeptide (PINP)

With the increasing of elderly population in the world, bone metabolism related diseases have attracted more and more attention. Biomarkers of bone turnover have been important as clinical indicators assist in the diagnosis of bone diseases, such as Paget's disease, rickets, osteomalacia, osteoporosis etc. Additionally, these biomarkers play a significant role in monitoring therapeutic interventions.

Procollagen type I N-propeptide (PINP) is a trimeric peptide with a molecular mass of about 35 000 kDa. It consists of two type I procollagen α1 chains and one procollagen-α2 chain. During the bone formation, type I procollagen's amino and carboxy-terminal regions are cleaved off by specific proteases during its conversion to collagen. During this process, N-terminal extension propeptide is released into the blood stream (1).

A1-PINP and PINP antibodies as diagnostic markers

The International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry (IFCC) bone marker standards working group have identified PINP and Cross-linked C-telopeptide of type 1 collagen (CTX, CrossLaps) to be the reference markers of bone turnover markers for bone formation and bone resorption respectively (2). Serum PINP has been endorsed by the National Bone Health Alliance (NBHA) in the US (3). These biomarkers of bone formation and resorption are critical clinical tools for assessing and monitoring bone metabolism. Comparing to the standard way of measuring the bone density, these markers provide a more direct and quicker way to see the result of intervention. In the management of osteoporosis, the main utility of serum PINP is for monitoring osteoporosis therapy.

Serum PINP has very low circadian and biological variation, barely affected by food intake, and is very stable during storage in serum. It has been shown that either serum or EDTA plasma sample may be used for PINP measurement, and the results treated interchangeably as they are equivalent. PINP in both EDTA plasma and serum is stable for at least 24 h at room temperature and for 5 days at 4°C (4).

Oligomeric forms of PINP in human blood

It is believed that major part of serum PINP constitutes by trimeric form in addition to monomeric form. Trimeric form is represented by 2 α1 chains and 1 α2 chain whereas monomeric form is a single α1 chain. Current immunoassays could measure either so called total PINP (both oligomeric and monomeric forms of PINP) or "intact" PINP which is essentially a trimer form of PINP only (5). Trimeric and monomeric forms of PINP are believed to be cleared from bloodstream by different mechanisms – trimeric form is cleared by liver whereas monomeric form is removed by kidney. Patients with compromised kidney function (i.e., terminal chronic kidney disease) may have spuriously elevated level of total PINP due to build-up of monomeric form of PINP which is not cleared in proper way (6).

CLINICAL UTILITY

- **Assessment and monitoring of osteoporosis therapy**
- **Diagnostic assistance and treatment monitoring for monostotic Paget's Disease**

Antibody pairs recommendations for immunoassay development

Hytest offers five new monoclonal antibodies (MAbs) for the development of highly sensitive, quantitative PINP immunoassays. These MAbs are specific for human PINP and are capable of recognizing all oligomeric forms of human PINP (total PINP). The performance of the MAbs was evaluated using chemiluminescent sandwich immunoassay. The capture MAb was conjugated with magnetic particles, and meanwhile the detection MAb was conjugated with alkaline phosphatase (ALP). The recommended capture-detection pairs for human PINP sandwich immunoassays are shown in Table 1. Recombinant A1-PINP (recA1-PINP) has been used for immunization of animals and for selection of antibody-producing cells. Antibodies developed could constitute several MAb pairs detecting recA1-PINP with high sensitivity (Fig. 1).

A1-PINP, recombinant, human

Additionally, we also provide recombinant A1-PINP antigen (recA1-PINP) to be used as a standard or calibrator for the immunoassay development. Recombinant protein corresponding to the sequence 23-161 of human collagen alpha-1(I) chain (UniProt ID P02452) was developed in mammalian cell line. His6-tag was added to the protein C-terminus to facilitate purification. A1-PINP was purified to >92% homogeneity as indicated by gel electrophoresis (Fig.2). Metal-affinity chromatography was used as a purification method. Hytest's recA1-PINP exists in two oligomeric forms as indicated by gel-filtration (Fig.3). Since monomers are linked non-covalently in the trimers of PNIP (5) there is a dynamic equilibrium of different oligomeric forms and their ratio at given time point depends on various factors such as concentration and pH, post-translational modifications may also play a role.

To study the size distribution of recA1-PINP in comparison to native PINP in human serum we did gel-filtration studies with human serum with or without added rec A1-PINP. Immunoreactivity in fraction was detected in time-resolved fluoroimmunoassay using W543-W188 MAb pair (Fig.4).

Immunoreactivity peaks of rec A1-PINP added to serum and native endogenous serum PINP as measured by MAb pair W543-W188 are overlapping indicating that recA1-PINP performs similarly to native PINP in immunoassay.

Table 1.
Recommended MAb combinations for the detection of human PINP.

Capture	Detection
W188	W509
W543	W509
W188	W555
W509	W510
W543	W188

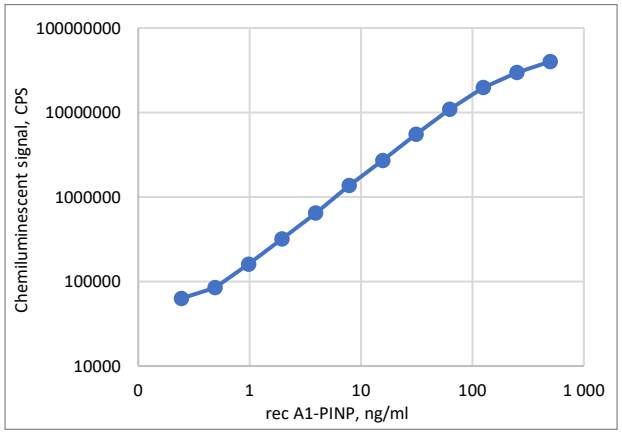

Figure 1.
Calibration curve for MAb pair W509-W510 taken in automated CLIA assay. Capture MAb was conjugated with magnetic particles, detection MAb was conjugated with alkaline phosphatase. RecA1-PINP has been used as a calibrator.

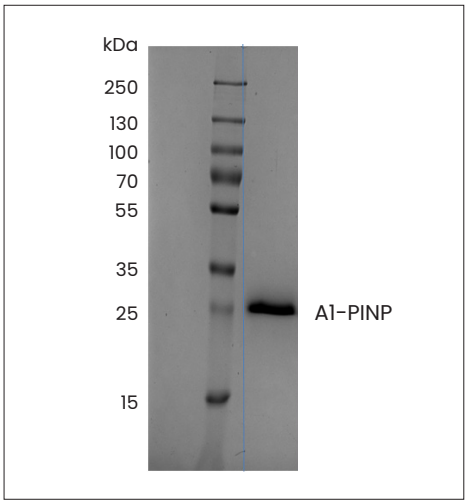

Figure 2.
SDS-gel electrophoresis in reducing conditions of purified A1-PINP recombinant. 3 µg of protein was loaded onto gel.

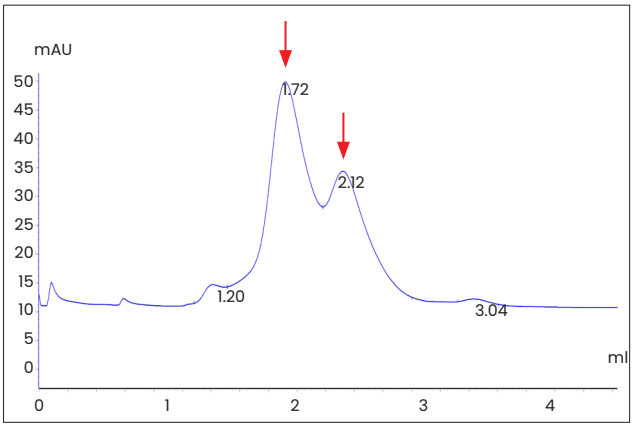

Figure 3.
Gel-filtration of purified recA1-PINP on Superdex 200 5/150 column. Blue line represents absorbance at 280 nm, red arrows indicate positions of oligomeric (trimeric) and monomeric forms of recA1-PINP.

Oligomeric composition in normal human serum

To further study the oligomeric composition of PINP in normal human serum, serum samples from apparently healthy donors were subjected to gel-filtration and immunoreactivity in fractions was detected in automatic particle-based CLIA using MAb pair W509-W543 (Fig.5)

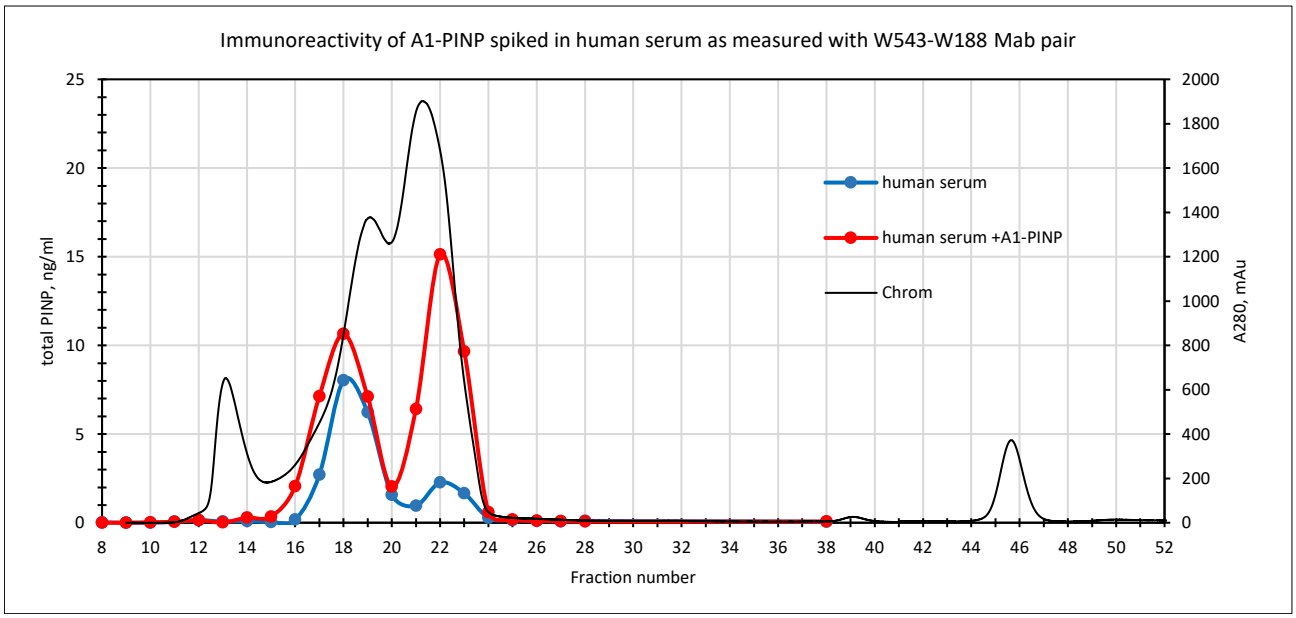

Figure 4.
Detection of native PINP in human serum specimen and recA1-PINP spiked in the same human serum specimen by MAb pair W543-W188 in time-resolved fluoroimmunoassay (DELFIA). Proteins in human serum were resolved by gel filtration. For fluoroimmunoassay, W543 was pre-adsorbed onto plate surface, W188 was conjugated with stable Eu^{3+} chelate.

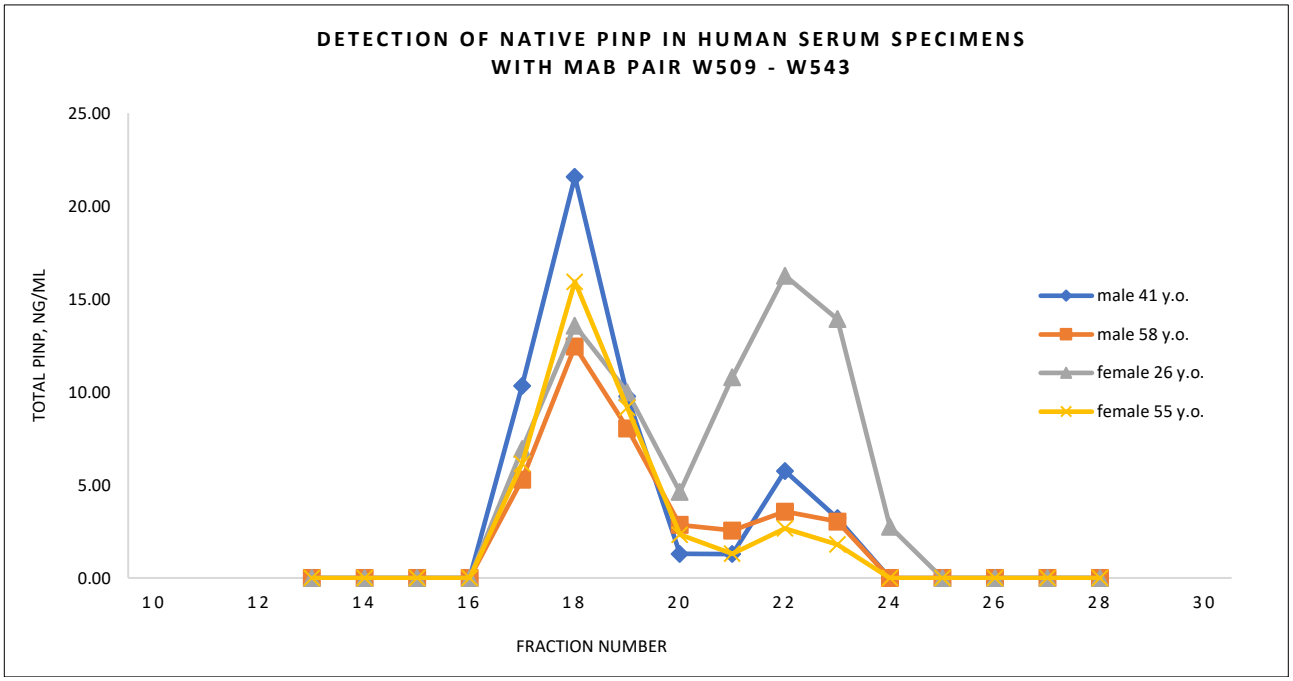

Figure 5.
Detection of native oligomeric forms of PINP in different serum specimens using automatic CLIA assay with MAb pair W509-W543. Capture MAb was conjugated with magnetic particles, detection MAb was conjugated with alkaline phosphatase. RecA1-PINP has been used as a calibrator.

This very small proof-of-concept study demonstrates that monomeric form of human PINP exists in substantial amount in serum of apparently healthy individuals and it's ratio to oligomeric form of PINP might decrease with age. There are indications in the literature though that monomeric form of PINP might be increased in patients with compromised kidney function due to aberrant clearance of this form of human PINP (5,6). We showed that in apparently healthy adults' monomer of human PINP could be present in significant amount whereas kidney function of these donors is not compromised.

The correlation of serum PINP concentrations

To check whether concentrations of native total PINP measured in human serum with Hytest MAb pairs are in agreement with concentrations measured in the same specimens by Roche Elecsys total PINP assay we conducted a comparison study (N=20) and established that these concentrations are correlated (Fig.6).

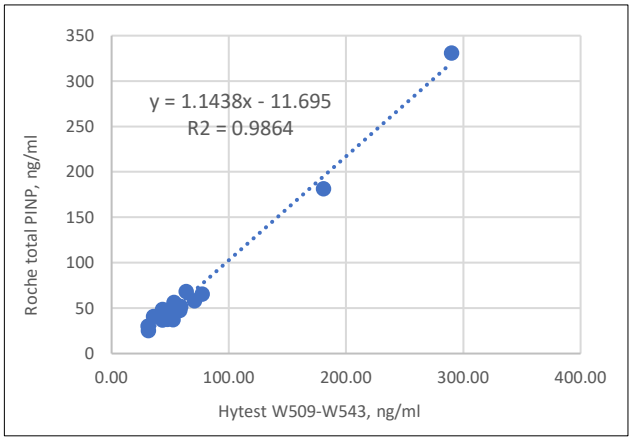

Figure 6. Correlation study of Roche Elecsys total PINP assay and Hytest MAb pair W509-W543 used in automated CLIA assay. Capture MAb was conjugated with magnetic particles, detection MAb was conjugated with alkaline phosphatase. RecA1-PINP has been used as a calibrator.

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Monoclonal anti-N-terminal procollagen I (PINP)	4PIA7	W188	IgG1	CLIA, mouse monoclonal antibody
		W509	IgG1	CLIA, recombinant chimeric antibody
		W510	IgG1	CLIA, recombinant chimeric antibody
		W543	IgG1	CLIA, recombinant chimeric antibody
		W555	IgG1	CLIA, recombinant chimeric antibody

ANTIGEN

Product name	Cat. #	Purity	Source
Procollagen type I N-propeptide (PINP), human, recombinant	8PIN7	>92%	Recombinant

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