



PIIINP ELISA KIT

Matching requested clinical and analytical performances

APPLICATION NOTE

ABSTRACT PIIINP is the recommended biomarker for monitoring methotrexate-induced hepatotoxicity in Psoriatic patients, where the degree of liver fibrosis correlates well with the amount of PIIINP (1,2,3,4,5).

Due to the anti-inflammatory properties of methotrexate, this drug is used for the treatment of patients with rheumatoid arthritis and psoriasis. Low doses of methotrexate can accumulate over time to reach toxic levels of 1.5g, generating liver damage and inducing liver fibrosis.

Guidelines recommend measuring the PIIINP blood concentration once before methotrexate treatment, and then continued sample testing twice a year during treatment.

The PIIINP blood concentration is low (<10 ng/mL), and therefore requires sensitive assays.

The clinical sensitivity of PIIINP is 81% and its specificity is only 62% , as the marker is not organ specific but linked to fibrotic processes. PIIINP could be elevated in all pathologies presenting organ fibrosis, like alcoholic liver disease, viral hepatitis, or primary biliary cirrhosis, as well as in a growing child linked to bone metabolism (9).

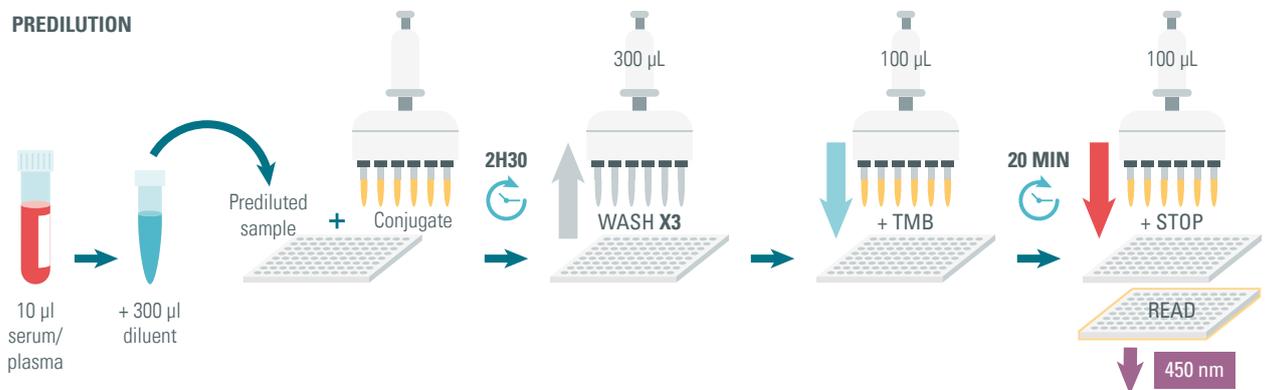
While liver biopsy remains the gold standard but involves an invasive procedure (4,5,6,7,8), measurement of PIIINP in blood is an important alternative for patient follow-up. Liver biopsy is however recommended if the patient shows continuous elevated concentrations of PIIINP (9).

Studies have shown that PIIINP is one of the most prominent and sensitive biomarkers for the clinical application mentioned (6,10,11,12,13).

Here, we will show that Cisbio's PIIINP ELISA monitors PIIINP blood levels reliably and accurately.

ASSAY WORKFLOW

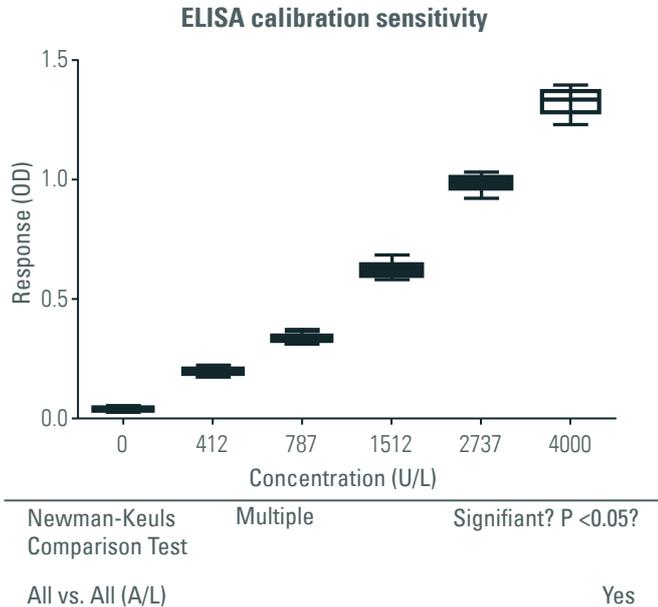
PREDILUTION



PRINCIPAL CHARACTERISTICS OF THE ASSAY:

- 2 monoclonal antibodies in a sandwich assay
- 1step incubation
- Automation friendly (DS2, Gemini, Tecan, ...)
- No cross reactivity with other collagen, including Col 1 fragment
- Small sample volume needed (10 µL)
- Plasma/EDTA (recommended) or Serum are possible.

PIIINP ELISA CALIBRATION SENSITIVITY



Newman-Keuls Multiple Comparison test and box plots were based on the accumulated responses (n=8) for each ELISA calibrator concentration. The diagram shows that all calibrators could be significantly differentiated. (Data provided by Karolinska University Hospital (1U/L= 7.8.10-3 µg/L)

PIIINP ELISA INTER/INTRA ASSAY PRECISION

Precision - within-series	Mean	SD	CV _{intra}	n
LEVEL 1 (human serum, pool)	5,17	0,11	2,2%	31
LEVEL 2 (human serum, pool)	13,4	0,32	2,4%	31
LEVEL 3 (human serum, pool)	25,5	0,92	3,6%	31

Precision - between-series	Mean	SD	CV _{inter}	n
LEVEL 1 (human serum, pool)	5.32	0.35	6.5%	8
LEVEL 2 (human serum, pool)	13.10	1.05	8.0%	8
LEVEL 3 (human serum, pool)	24.96	1.81	7.3%	8

The intra-assay (within-run) variation was determined by 31 measurements of 3 serum samples covering the whole measuring range of the calibration curve.

The Inter-assay (between-run) variation was determined using 3 serum samples measured in 8 runs in duplicate.

Excellent reproducibility of the assay was found both within and between runs, ensuring high-confidence data.

DETECTION LIMIT AND QUANTIFICATION LIMIT

LOD 2 σ

0.036 µg/L

The limit of detection was calculated using 30 replicates of Calibrator "0" at 95% CI

LOQ 12.5%CV_{inter}

<< 2 µg/L

The limit of quantification was calculated using 9 EDTA plasmas in duplicate, measured through 8 different runs.

These data demonstrate the suitability of the sensitivity for a good discrimination of pathological samples (cut-off at 11.7 µg/L).

RECOVERY PERFORMANCES

EDTA-plasma sample	Expected value (U/L)	Measured value (U/L)	Recovery
Level 1	716	684	93 %
Level 2	1027	1011	98%
Level 3	1505	1438	95%

Serum sample	Expected value (U/L)	Measured value (U/L)	Recovery
Level 1	713	709	99 %
Level 2	1027	999	96%
Level 3	1512	1435	94%

These data provided by Karolinska University Hospital show that recoveries for PIIINP were within the criteria set by the lab (90%-110%).

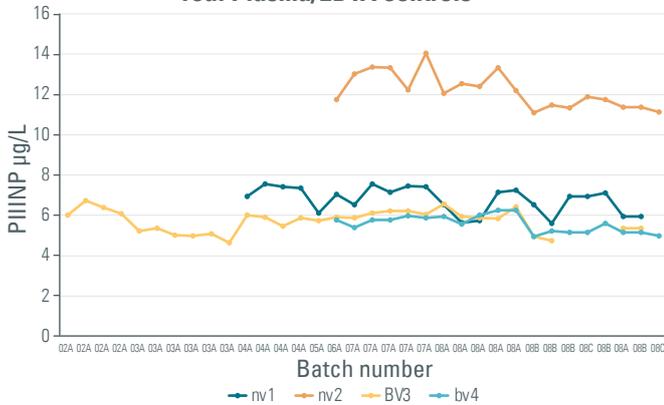
The results demonstrate the capability of the kit to accurately measure the analyte without any matrix effect.

Dilution linearity test (not shown in this document) is also good, based on 4 elevated Plasma/EDTA samples (1, 1/2, 1/4, 1/8).

Intercept	-0.11<x<0.3
Slope	0.99<x<1.03
r2	> 0.998

BATCH TO BATCH REPRODUCIBILITY:

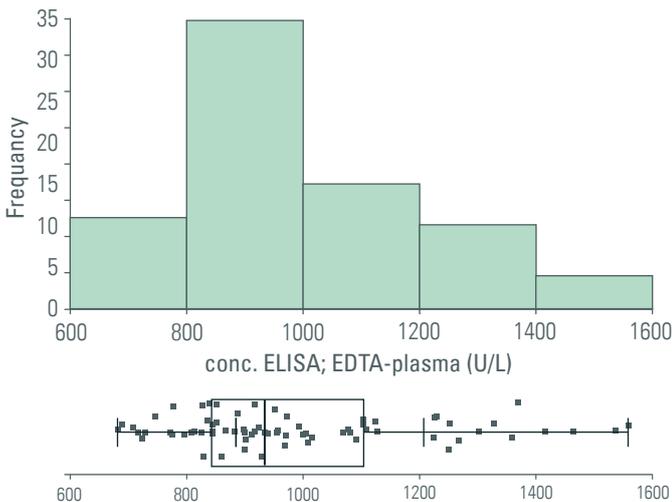
Batch to batch reproducibility using real Plasma/EDTA controls



Because the clinical use is in the follow-up of psoriatic patients, reproducibility of the assay over time is mandatory. For this reason, four real sera were continuously monitored for the different batches produced by Cisbio Bioassays to check the reproducibility of the assay over time. The figure shows a very good batch to batch reproducibility, allowing clinicians to ensure accurate liver activity monitoring for the patient.

NORMAL VALUES

Reference (normal) range: ELISA (EDTA-plasma)

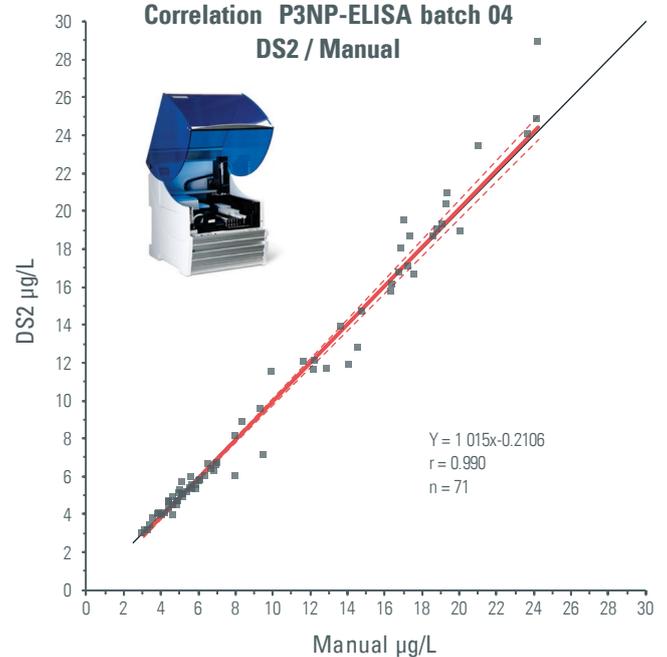


	Min.	1 st quartile	Median	3 rd quartile	Max.	Mode
conc. ELISA; EDTA-plasma (U/L)	681	843	933	1104	1561	912

Quantile	conc. ELISA; EDTA-plasma (U/L)
0.050	717
0.950	1412

These data provided by Karolinska University Hospital were generated from 75 plasma EDTA of presumably healthy patients. The cut-off was determined at 95% CI at 1500 U/L (11.7 µg/ml). It is mandatory for each lab to generate its own normal values and cut-off.

AUTOMATION: EXAMPLE ON DS2 MACHINE



As automation is more and more a key criterion for clinical labs, 71 plasma EDTA samples were checked in parallel in manual and automated versions of the kit. The excellent correlation demonstrates the ability of the kit to be automatized (Cisbio Bioassays).

ROBOTIC AT KAROLINSKA UNIVERSITY HOSPITAL



Ready-to-use reagents were put into predefined cups (A) and samples were loaded onto the robot. An 8-channel pipettor (B) was used to transfer sample and reagent material between tubes/cups and the 96-well plate (C). The gripper (D) was used to move the plate into different sections. Each section was specifically dedicated: sample preparation (8-channel pipettor), plate incubation (E), plate washing (F), or UV/Vis spectrophotometry (G).

The ease of automation of Cisbio's PIIIINP-ELISA kit was one of the main reasons for the Karolinska University Hospital's switch from RIA to ELISA.

CONCLUSION

The PIIINP ELISA is a very robust kit, perfectly adapted to precise and accurate monitoring of PIIINP in blood samples.

Some reference labs, like the Karolinska University Hospital in Stockholm mentioned in this Application Note, are already using the kit routinely to monitor psoriatic patients under methotrexate treatment.

Batch to batch reproducibility is mandatory for patient follow-up, which is why Cisbio has made considerable – and successful - efforts to minimize variations.

The kit's robustness also enables an easy conversion from manual to automated use.

BIBLIOGRAPHY

1. *Hiramatsu et al, (1995) J.Hepatol 22(2):135-142.*
2. *Läkemedelverket, (2006) Med.Prod.Agency 17(5):2415.*
3. *Pathirana et al, (2009) J.E.A.D.V 23 suppl 2: European S3 Guidelines on the systemic treatment of psoriasis vulgaris.*
4. *Aithal, (2011) Nat Rev Rheumatol 7(3): 139-150.*
5. *Bath et al, (2014) J Dig Dis 15(10): 517-524.*
6. *Zachariae et al, (2001) Bri J Dermatol 144(1): 100-103.*
7. *Khan et al, (2006) Postgraduate Med J 82(967): 353-354.*
8. *Sharma et al, (2014) World J of Gastroentero 20(45): 16820-16830.*
9. *Läkemedelverket (2011)*
10. *Oh et al, (2001) Current Gastroenterology Reports 3(1): 12-18.*
11. *Boffa et al, (1996) Br J Dermatol 135(4):538-544.*
12. *Chalmers et al (2005) Br J Dermatol 152(3): 444-450.*
13. *Maurice et al, (2005) Br J Dermatol 152(3): 451-458.*

FOR MORE INFORMATION

Europe and other countries +33(0)466 796 705 U.S. and Canada 1-888-963-4567 China +86 10 8080 9288

Visit www.cisbio.com to find a list of our regional distributors