

ELISA FOR THE MEASUREMENT OF URINARY VANIN-1 AS POTENTIAL NEW BIOMARKER FOR KIDNEY INJURY

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SUMMARY AND CONCLUSION

Features of the Vanin-1 (urine) sandwich ELISA:

- One-step ELISA
- Optimized for urine samples
- Characterized antibodies
- Assay range: 0 – 1200 pmol/l
- Validated according to FDA/ICH/EMA guidelines
- Good sample stability
- Sample values provided

INTRODUCTION

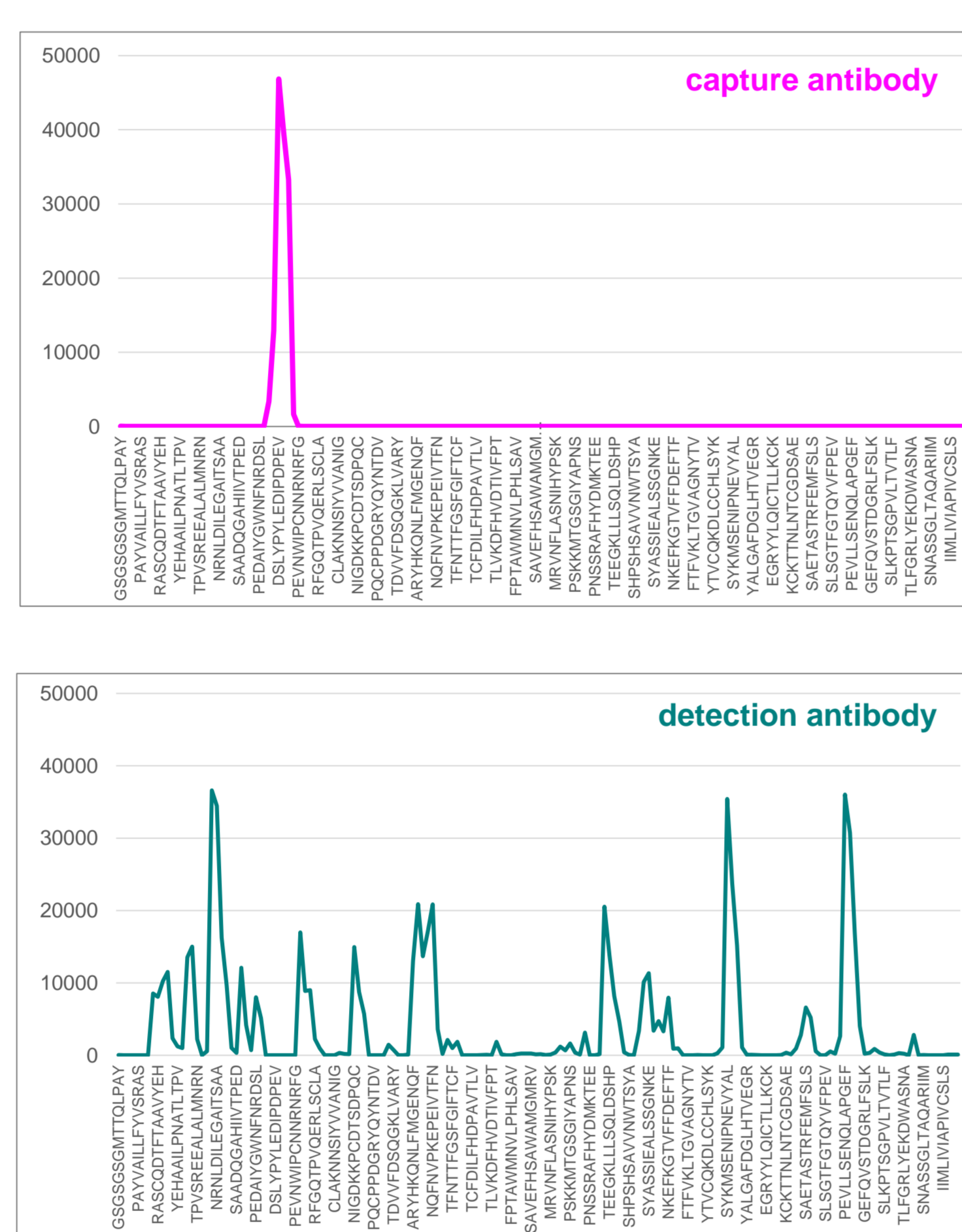
Vascular Non-Inflammatory Molecule 1 (Vanin-1) is a GPI-anchored ectoenzyme catalyzing the hydrolysis of pantothenic acid (vitamin B5) and cysteamine. It is involved in the regulation of oxidative stress and inflammation. The highest expression levels of Vanin-1 were observed in kidney tubular epithelial cells, especially under conditions of oxidative stress.

Vanin-1 released from injured renal cells could be detectable in urine. Indeed, recent studies indicate that urinary Vanin-1 may be an earlier biomarker for acute kidney injury, as well as for the detection of nephrotoxicant-induced renal injury, than established markers like NGAL and KIM-1.

To further study to potential of urinary Vanin-1 as biomarker for kidney injury, there is a need for a highly specific and sensitive assay that is optimized for the detection of Vanin-1 in human urine.

ANTIBODIES

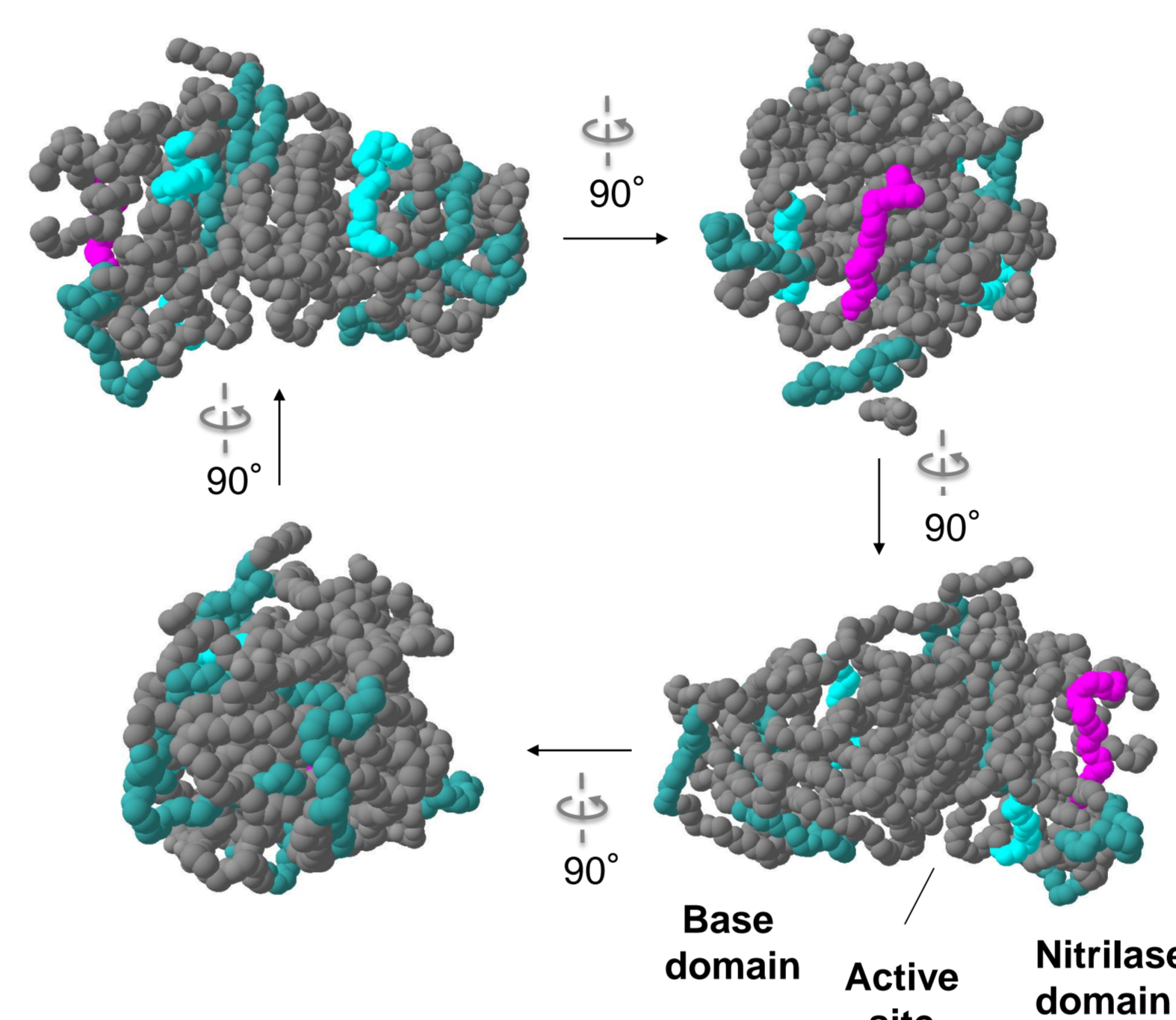
Epitope mapping of antibodies



Epitope mapping of peptide purified capture antibody (upper panel) reveals the linear epitope at position 96-104 with the sequence LEDIPDPEV.

The detection antibody (lower panel) has 12 main linear epitopes throughout the whole sequence.

Distribution of linear epitopes of antibodies in the Vanin-1 molecule



3D structure of human Vanin-1 with designated binding sites of both antibodies. The epitope of capture antibody (pink) is located in the nitrilase domain, whereas the detection antibody can bind throughout the whole molecule. Displayed are 4 different orientations of the molecule.

PROTOCOL

One-step ELISA

50 µl assay buffer
+ 10 µl sample / standard / control
+ 50 µl detection antibody
4 h at RT
5 x washing
100 µl substrate
30 min at RT
50 µl stop solution
Read OD at 450 nm

VALIDATION DATA

Validation according to FDA/ICH/EMA guidelines

Sensitivity	9.6 pmol/l (= 500 pg/ml)
Precision	Within-run (n=3): ≤ 5 % CV
Accuracy	+ 120 pmol/l: 81 % + 600 pmol/l: 93%
Parallelism	1+1: 94% 1+3: 92% 1+7: 86%
Dilution linearity	1+1: 94% 1+3: 91% 1+7: 80%

METHODS

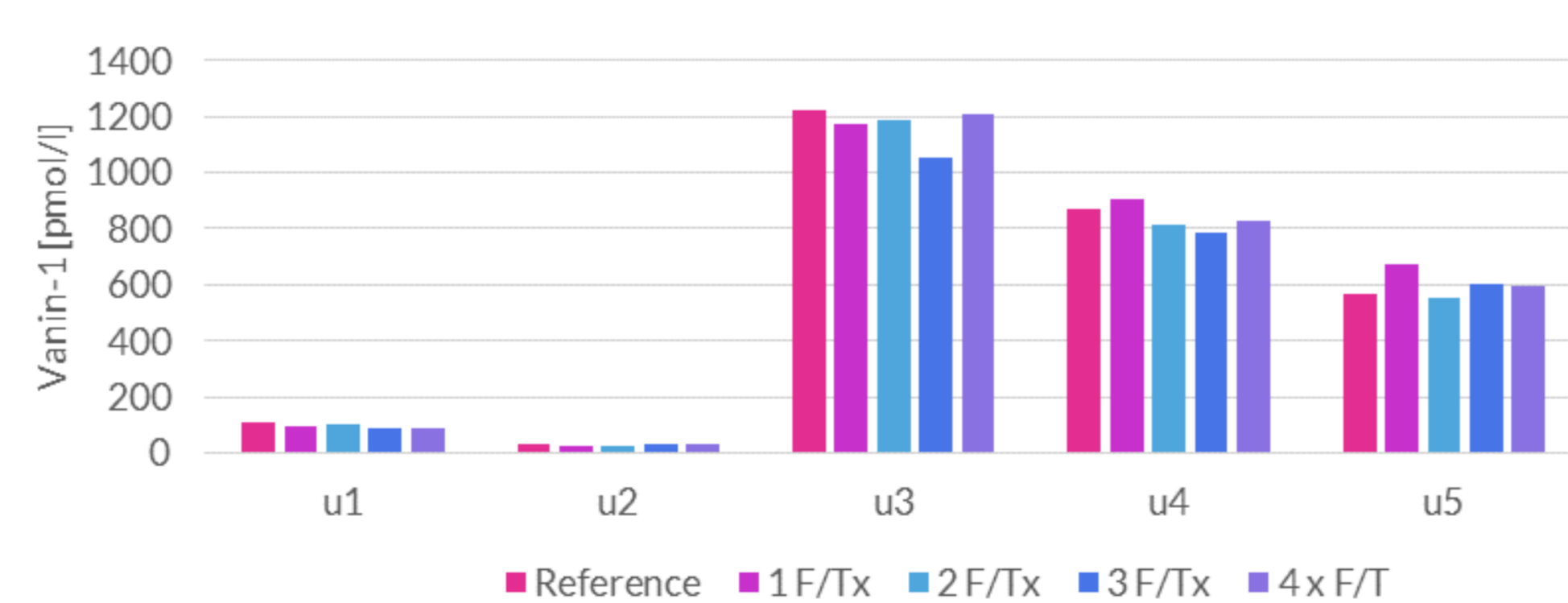
We developed a sandwich ELISA for the quantification of human urinary Vanin-1 based on a peptide-specific capture antibody and a polyclonal detection antibody. Both antibodies were characterized regarding their purity and specificity.

The ELISA was validated according to ICH/FDA/EMA guidelines, which includes the assessment of assay parameters like precision, specificity, parallelism, accuracy, and sample stability.

Vanin-1 concentrations were measured in urine samples from apparently healthy controls and subjects with impaired kidney function.

SAMPLE STABILITY

Freeze-thaw stability

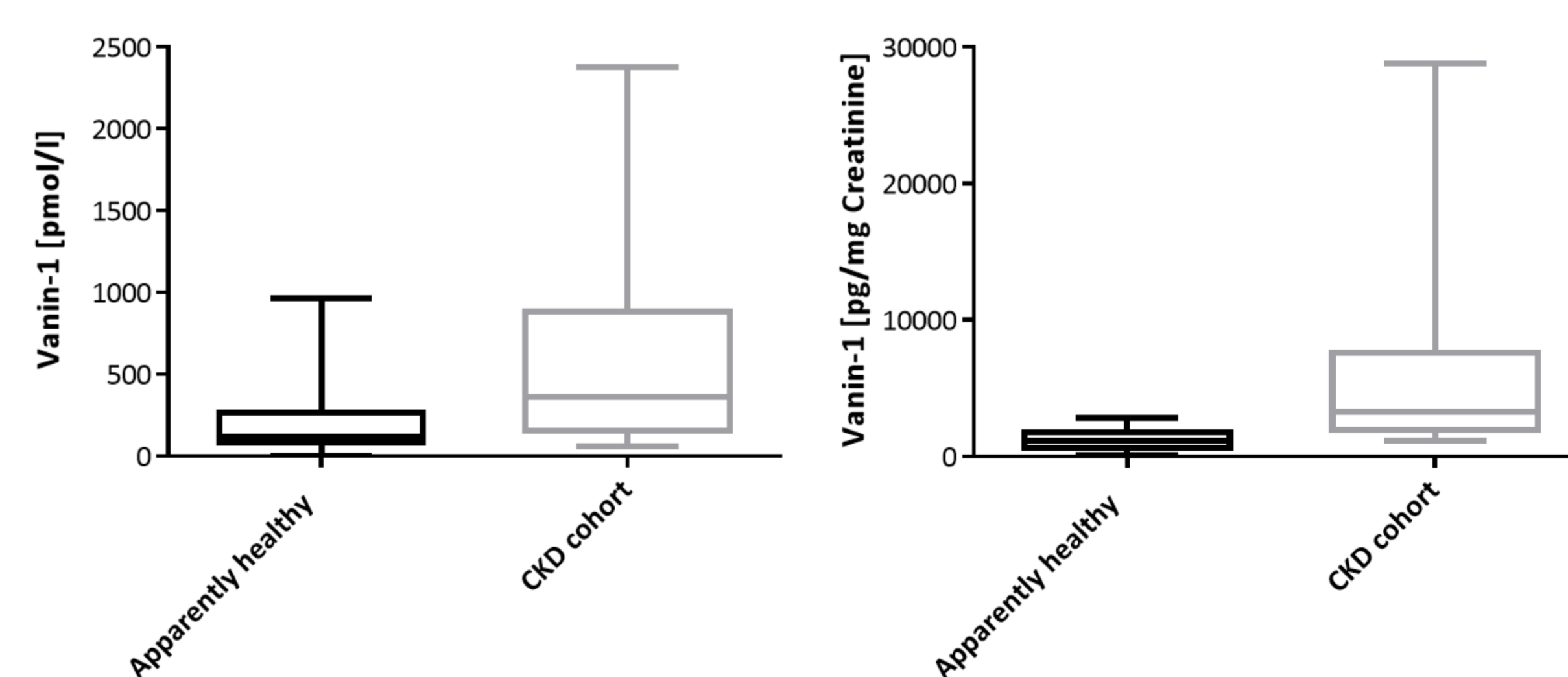


Freeze-thaw stability of endogenous Vanin-1 was tested by comparing Vanin-1 measurements in urine samples that had undergone four freeze-thaw (F/T) cycles. Samples can undergo at least four freeze-thaw cycles. The mean recovery of sample concentrations stressed by four freeze-thaw cycles is 96%.

Benchtop stability (data not shown) of endogenous Vanin-1 was tested in a panel of urine samples. Samples can be stored for at least three hours at room temperature as well as overnight at 4° C .

SAMPLE VALUES

Vanin-1 urine values in apparently healthy and diseased individuals



Vanin-1 values (left panel) in urine samples from apparently healthy individuals (n=27; median = 116 pmol/l) and individuals with kidney disease (CKD) (n=24; median = 360 pmol/l).

In addition, these values were converted from pmol/l into pg/ml (conversion factor: 1 pg/ml= 0.0192 pmol/l) and **normalized to Creatinine** values (right panel). Apparently healthy individuals: median = 1131 pg Vanin-1/mg Creatinine; CKD cohort median = 3289 pg Vanin-1/mg Creatinine.

LITERATURE

- 1) Hosohata K., Washino S., Kubo T., Natsui S., Fujisaki A., Kurokawa S., Ando H., Fujimura A., Morita T. (2016): Urinary vanin-1 as a novel biomarker for early detection of drug-induced acute kidney injury. Toxicology 359–360, 71–75.
- 2) Hosohata K., Ando H., Fujimura A. (2012): Urinary vanin-1 as a novel biomarker for early detection of drug-induced acute kidney injury. J Pharmacol Exp Ther 341(3):665-62

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