SP-278

VALIDATION OF A C-TERMINAL FGF23 MULTI-MATRIX SANDWICH ELISA FOR THE DETECTION OF FGF23 IN HUMAN SERUM AND PLASMA

Jacqueline Wallwitz[1], Brigitte Eichinger[1], Gabriela Berg[2], Elisabeth Gadermaier[1]

[1]The Antibody Lab GmbH, Vienna, Austria

[2]Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria

SUMMARY AND CONCLUSION

Special features of the validated multi-matrix sandwich ELISA for the detection of C-terminal FGF23

- **Characterized antibodies**
- **Defined analyte** (C-terminal FGF23)
- **Serum and plasma** as sample matrix
- **Serum based standards** (0-20 pmol/l)
- **High sensitivity** (detection limit: 0.08 pmol/l)
- **High specificity** (>93%)
- **Precision** (<12% CV)
- **Accuracy** (>89%)
- **Dilution linearity** (100-108%)

We could show that C-terminal FGF23 multi-matrix ELISA may be a reliable tool to measure C-terminal FGF23 in serum as well as in plasma samples.

INTRODUCTION

Fibroblast growth factor 23 (FGF23) is secreted by osteoblasts and osteocytes and mainly regulates phosphate homeostasis and calcitriol levels.

The bioactive intact FGF23 contains 251 amino acids and is glycosylated and phosphorylated. Its activity is mediated by binding to FGFR/Klotho receptor complex at the target cell surface. Intact FGF23 is cleaved between Arg179 and Ser180 to an N-terminal and a C-terminal fragment.

Several studies revealed that FGF23 concentrations are increased in chronic kidney disease, oncogenic osteomalacia and several rare hereditary disorders. Most of these measurements were performed by using immunoassays, which detect only intact (intact FGF23 ELISA) or both intact and C-terminal fragments (C-terminal FGF23 ELISA).

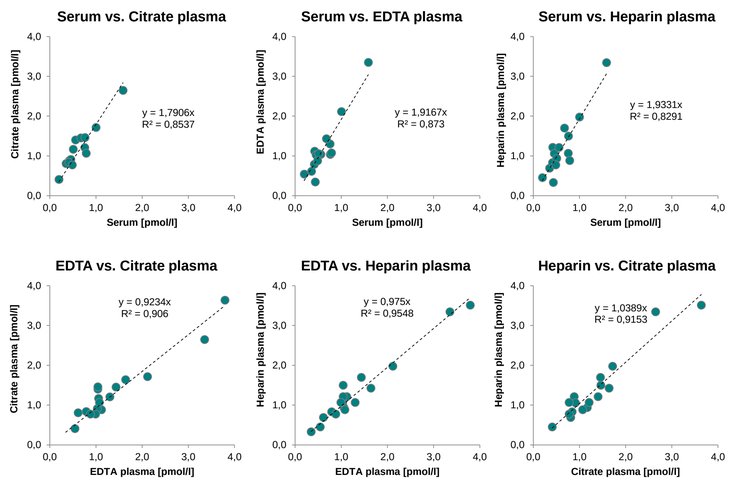
METHODS

Here, we show the validation and characterization of a C-terminal multi-matrix FGF23 ELISA. Epitopes of both polyclonal antibodies were analyzed by overlapping linear peptides spotted to a microarray and also determination of binding kinetics with biolayer interferometry was performed.

The assay was validated according to standard quality guidelines with a special focus on matrix comparison (serum and EDTA, heparin and citrate plasma) and analyte stability.

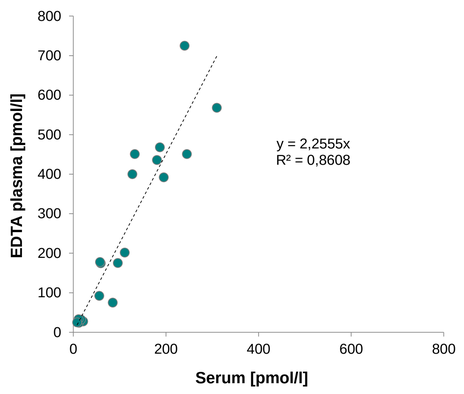
MATRIX COMPARISON

Very good correlation between different sample matrices of apparently healthy subjects



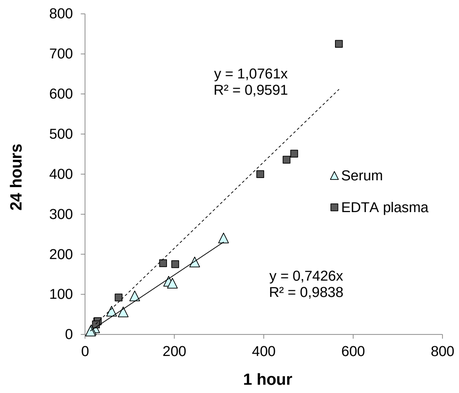
Sample matrix comparison (serum; EDTA, citrate and heparin plasma) of 18 apparently healthy volunteers. Correlation coefficient for each comparison is stated and is at least R^2>0.85. Plasma samples show higher concentration than serum samples.

Very good correlation between serum and plasma of CKD patients



Sample matrix comparison (serum and EDTA plasma) of samples from 20 CKD patients. FGF23 concentration in plasma samples is higher, but the correlation between both matrices is very good with R^2>0.86.

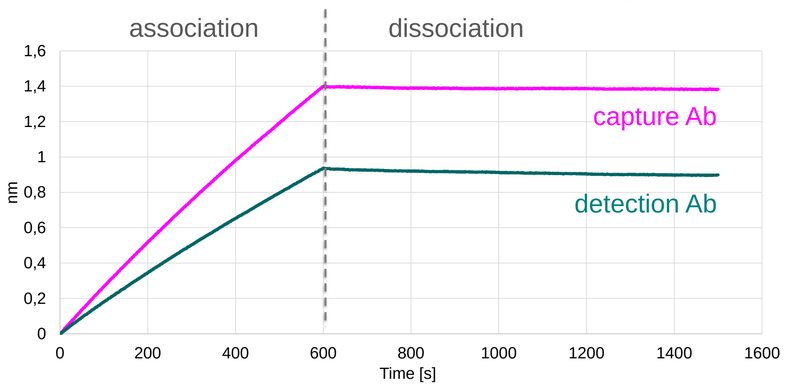
Whole blood stability of C-terminal FGF23 in plasma better than in serum



Whole blood stability of serum and EDTA plasma of 10 CKD patients. Whole blood was kept at room temperature for 1 hour (x-axis) or 24 hours (y-axis) before serum or EDTA plasma were prepared. Stability of C-terminal FGF23 in whole blood is better for plasma than for serum.

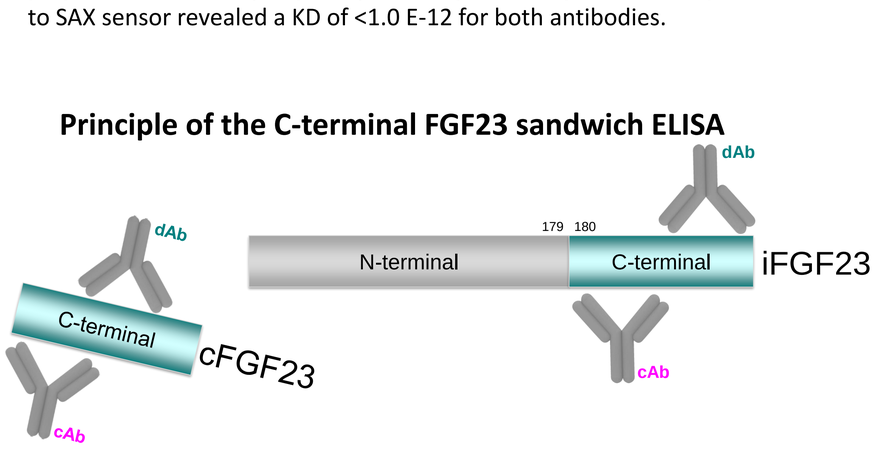
ANTIBODY AND ELISA CHARACTERISTICS

Both ELISA antibodies bind to FGF23 with high affinity



Biolayer interferometry measurements of polyclonal capture (pink) and polyclonal detection (turquoise) antibody to biotinylated FGF23 protein coated to SAX sensor revealed a KD of <1.0 E-12 for both antibodies.

Principle of the C-terminal FGF23 sandwich ELISA



The **C-terminal FGF23 ELISA** detects the C-terminal part of intact FGF23 (iFGF23) and the C-terminal fragment (cFGF23) as well. Both forms are circulating in serum and plasma samples. The capture antibody (cAb, pink) and the detection antibody (dAb, turquoise) have binding sites at the C-terminal FGF23.

The ELISA fulfills all validation requirements

ASSAY PARAMETERS	Matrix (n)	Mean [%]	Range [%]
SPECIFICITY			
Competition	Serum (7)	96	77-100
	ETDA (4)	94	93-99
	Heparin (4)	93	88-96
DILUTION LINEARITY			
1:2	Serum (9)	105	93-113
	EDTA (4)	103	67-127
	Heparin (10)	102	92-113
	Citrate (5)	102	94-106
1:4	Serum (9)	100	89-126
	EDTA (4)	103	69-120
	Heparin (10)	106	93-117
	Citrate (5)	106	93-123
1:8	Serum (9)	108	91-124
	EDTA (4)	106	68-124
	Heparin (10)	104	90-132
	Citrate (5)	101	93-118
SPIKE RECOVERY			
Lower range (5 pmol/l)	Serum (13)	96	84-108
	ETDA (7)	97	73-123
	Heparin (8)	101	68-161
	Citrate (7)	100	61-132
Upper range (10 pmol/l)	Serum (13)	89	60-103
	EDTA (7)	94	77-114
	Heparin (8)	92	69-118
	Citrate (7)	90	58-107

C-terminal FGF23 ELISA characteristics, whereas specificity was determined by adding at least 5-fold molar excess of capture antibody as competitor. For dilution linearity samples were diluted with assaybuffer. Accuracy was determined by spiking of two concentration (lower and upper range) of recombinant C-terminal FGF23.

LITERATURE

Erben RG, Andukhova O (2017): FGF23-Klotho signalling axis in the kidney. Bone 100:62-68

Smith ER, Cai MM, McMahon LP, Holt SG (2012): Biological variability of plasma intact and C-terminal FGF23 measurements. J Clin Endocrinol Metab 97(9):3357-65

Smith ER, McMahon LP, Holt SG (2014): Fibroblast growth factor 23. Ann Clin Biochem 51(Pt2):203-27

CONTACT & COI

Gabriela Berg: gabriela.berg@bmgrp.com

Jacqueline Wallwitz: j.wallwitz@theantibodylab.com

We have no relevant financial relationship to disclose any COI for this research presentation within the period of 36 months.