Novel ELISA for the quantification of human leucine-rich α-2 glycoprotein (LRG) in serum and plasma

Elisabeth Gadermaier[1], Jacqueline Wallwitz[1], Gabriela Berg[2], Gottfried Himmler[1]

[1]The Antibody Lab GmbH, Vienna, Austria

[2]Biomedica Medizinprodukte GmbH, Vienna, Austria

The Antibody Lab

BIOMEDICA

SUMMARY AND CONCLUSION

Special features of the LRG sandwich ELISA

- **High precision**
- **Characterized antibodies**
- **Wide assay range**
- **Includes: 7 standards, 2 controls**
- **Correlates well** with other assays
- **Full validation package**
- **For serum and plasma**
- **Good sample stability**

We could show that the LRG ELISA (Biomedica, BI-LRG) is a reliable and sensitive tool to specifically measure human LRG in serum and plasma samples.

INTRODUCTION

Leucine-rich α-2 glycoprotein 1 (LRG) is a highly glycosylated 38 kDa protein. It belongs to the leucine-rich repeat protein family whose members are involved *e.g.* in protein-protein interactions, cell signaling, or cell adhesion.

It is described as proangiogenic factor and increased levels are reported in various diseases where neovascularization is involved. For instance, it was shown that LRG induced by TNF-α promotes angiogenesis that then contributes to aberrant bone formation in the subchondral bone of osteoarthritis patients. A recent study highlights the clinical usefulness of the inflammatory marker LRG for the evaluation of disease activity in rheumatoid arthritis. Furthermore, LRG may also serve as a biomarker for rheumatoid arthritis disease activity during IL-6 blockade treatment.

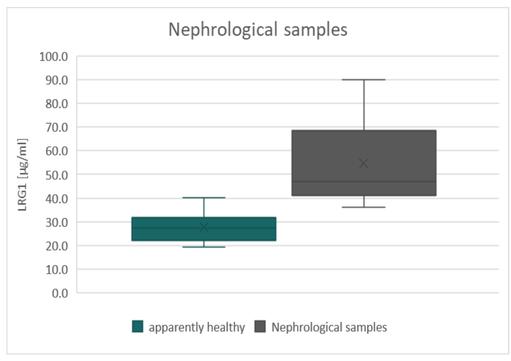
METHODS

Here, we show the development, characterization and validation of a new LRG ELISA (cat. no. BI-LRG, Biomedica, Vienna, Austria).

The assay is optimized and validated for human serum and plasma samples according to international quality guidelines. Urine and cell-culture supernatants are compatible with this ELISA.

Assay performance, sample matrix comparison as well as sample measurements of apparently healthy and diseased human subjects were performed.

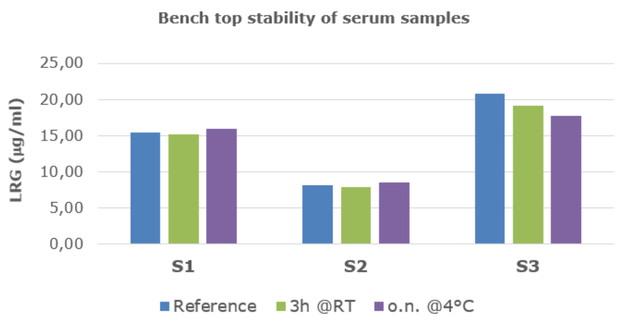
LEUCINE-RICH ALPHA-2-GLYCOPROTEIN (LRG) VALUES IN DISEASE PANELS



		LRG [µg/ml]		
Samples	n	Mean	Range	Median
Apparently healthy controls	18	27.7	19.2 - 40.2	27.5
Rheuma cohort	18	53.5	33.0 - 79.0	51.0
Arthrose cohort	16	39	12.0 - 89.0	33.0
Kidney disease	16	54.7	36.0 - 90.0	47.0

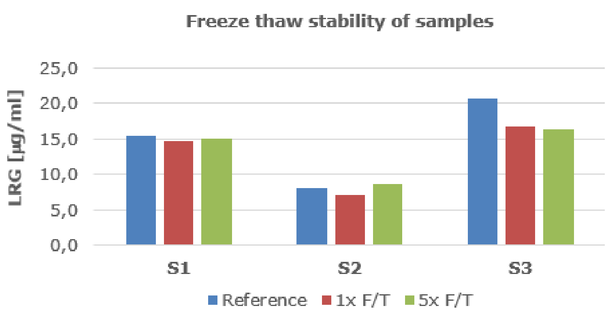
STABILITY OF LEUCINE-RICH ALPHA-2-GLYCOPROTEIN (LRG)

Benchtop stability of human samples



Benchtop stability of human serum samples containing endogenous LRG. Samples were stored for 3 hours at room temperature (rt) or overnight (o.n.) at 4° C and compared with the reference resulting in less than 4% measurement alterations.

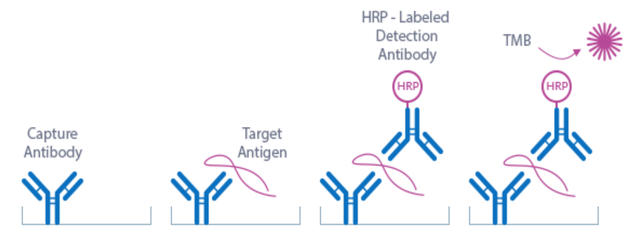
Freeze-thaw stability of human samples



Freeze-thaw stability (F/T) (x-axis) of three human samples containing endogenous serum LRG. Samples are stable for at least 5x freeze-thaw cycles.

LEUCINE-RICH ALPHA-2-GLYCOPROTEIN (LRG) ELISA CHARACTERISTICS

Principle of the detection of human LRG



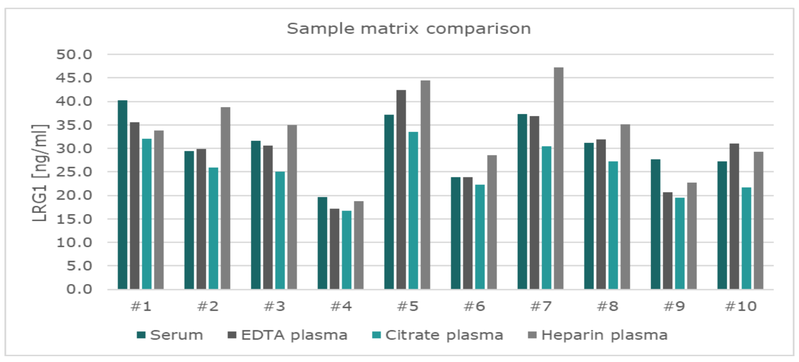
The LRG ELISA is a sandwich-based immunoassay. Antibodies were characterized by epitope mapping of linear epitopes with microarray technology and by the determination of binding kinetics with biolayer interferometry. Plates are coated with a peptide-specific polyclonal sheep anti-human LRG antibody binding to a linear epitope in the N-terminal region of LRG. Multiple linear epitopes recognized by the polyclonal detection antibody are distributed over the whole LRG sequence and are located in the N- and C-terminus, as well as within the leucine-rich repeats. Both antibodies bind to LRG with low dissociation rate constants.

LRG assay protocol

100 µl sample (prediluted) / standard / control (5µl sample volume required)
2h @ 18-26°C
5x washing
100µl detection antibody
2h @ 18-26°C
5x washing
100 µl substrate
30 min @ 18-26°C (dark)
50 µl stop solution read OD (450 nm)

Samples, standards (0 – 64 ng/ml) and controls are pipetted into the antibody coated plate. After a washing step the labelled detection antibody is incubated for 2 hours followed by an additional washing step. Thereafter, TMB substrate is added for 30 minutes, followed by stopping the reaction and OD measurement at 450 nm.

LRG ELISA validated for serum and plasma



LRG was measured in serum and plasma samples prepared from 10 apparently healthy donors. A mean CV values of 10% was observed between all measured sample matrixes which lies within the recommended validation quality guidelines. The assay can be used for all indicated samples matrices.

LITERATURE

- MicroRNA-497 elevation or LRG1 knockdown promotes osteoblast proliferation and collagen synthesis in osteoporosis via TGF-β1/Smads signalling pathway. Gu Z et al., J Cell Mol Med. 2020; 24(21):12619-12632.
- Leucine-rich alpha2-glycoprotein as the acute-phase reactant to detect systemic juvenile idiopathic arthritis disease activity during anti-interleukin-6 blockade therapy: A case series. Shimizu M et al., Mod Rheumatol. 2017; 27:833–837.
- Usefulness of Serum Leucine-Rich Alpha-2 Glycoprotein as a Disease Activity Biomarker in Patients with Rheumatoid Arthritis. Ha, You Jung et al., jkms. 2014; 29(9):1199–1204.

CONTACT

Gabriela Berg: gabriela.berg@bmgrp.com