P003

Highly sensitive quantification of human VEGF with a novel ELISA

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SUMMARY AND CONCLUSION

This new human VEGF immunoassay uses well-characterized antibodies to quantify all circulating VEGF isoforms (incl. $VEGF_{165b}$). The assay employs a recombinant capture antibody that binds within the receptor binding region of the molecule, which is conserved among all isoforms. Therefore, the assay only detects VEGF that is not bound to its receptors VEGF R1/ VEGF R2. Furthermore, Neuropilin-1, a VEGF co-receptor, does not interfere with this assay. In consequence, Neuropilin-1 free and bound VEGF forms are quantified. Linear epitopes of the detection antibody are concentrated within the N-terminal of the VEGF protein. We demonstrate that VEGF can reliably be measured in serum and plasma. Most importantly, sample signal of all apparently healthy individuals is above background. Assay characteristics as well as analyte stability meet international quality standards of acceptance. In conclusion, our novel VEGF ELISA provides a reliable and accurate tool for the quantitative determination of all circulating VEGF isoforms with high sensitivity.

BACKGROUND

Vascular endothelial growth factor A (VEGF or VEGF-A) is one of the most important regulators of vascular development and angiogenesis. It also plays critical roles in skeletal development as osteoblastic cells represent a major source of VEGF in the bone environment. Compromised bone repair and regeneration in many patients can be attributed to impaired blood supply; thus, VEGF represents a relevant therapeutic target.

Alternative splicing of VEGF mRNA gives rise to various isoforms with VEGF 165a being the most frequently expressed form with a molecular weight of 22.3 kDa (monomer). Measurement of low concentrations of circulating VEGF, especially in apparently healthy individuals, proves to be a challenge. Hence, there is a need for a highly sensitive assay that enables the reliable quantification of low concentrations of human VEGF.

METHODS

We developed a highly sensitive sandwich ELISA for the specific detection of human VEGF using high quality, well-characterized recombinant monoclonal and polyclonal anti-human VEGF antibodies. The linear epitopes of the polyclonal detection antibody were mapped with microarray technology. Analyte stability was determined and in accordance with ICH and EMEA quality guidelines, assay parameters like specificity, dilution linearity, and spike recovery were assessed.

ANTIBODY CHARACTERISTICS

Microarray of detection antibody

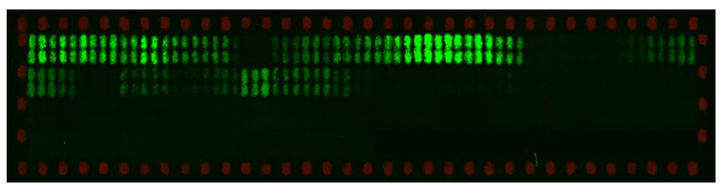

Fig. 1: High resolution epitope mapping of the polyclonal detection antibody on human VEGF. The canonical sequence of VEGF (P15692-1) was printed as 15mers with an 14 amino acid overlap in duplicates on a glass chip. Green fluorescent signals on the microarray illustrate binding of the polyclonal detection antibody to VEGF and corresponds to its epitopes. Red fluorescent signals mark the position of control peptides. The polyclonal detection antibody recognizes linear epitopes N-terminal of VEGF.

Antibody epitopes on human VEGF molecule

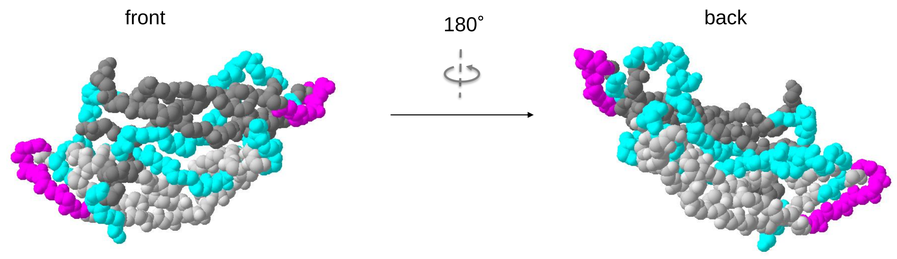

Fig. 2: 3D structure of human VEGF dimer (V14-K107, pdb 1BJ1) with antibody epitopes. The recombinant capture antibody recognizes a structural epitope (purple) in the conserved receptor binding-site of VEGF (shown as dimer, dark and light grey) and thus, specifically binds to all bioactive isoforms of VEGF. Linear epitopes (blue) of the detection antibody are concentrated in the first 120 amino acids of the VEGF molecule.

HUMAN VEGF ELISA

Matrix comparison

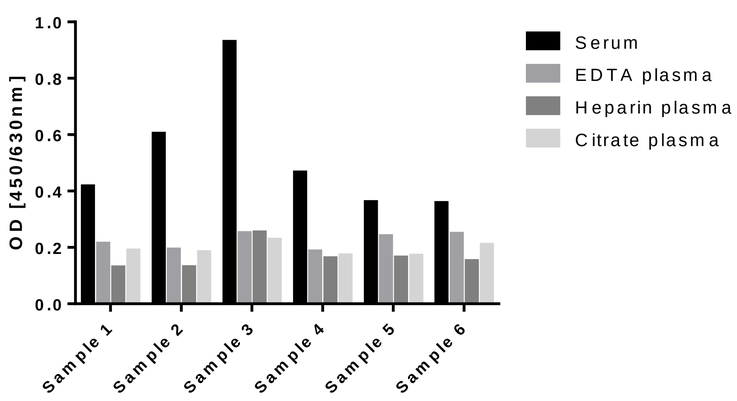

Fig. 3: Comparison of VEGF levels in different blood preparations. Plasma preparations of six apparently healthy blood donors show comparable VEGF levels among different plasma qualities. Serum shows significantly increased values.

VEGF-levels above background in apparently healthy subjects

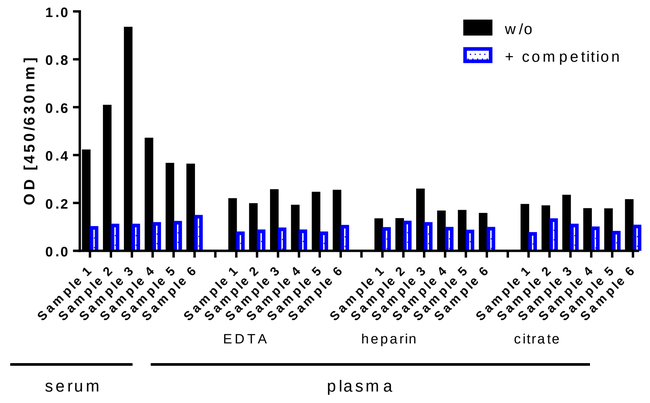

Fig. 4: Competition experiments with a 20-fold excess of capture antibody added to serum/plasma preparations of apparently healthy individuals demonstrate signal specificity of the test. Most importantly, these experiments highlight that VEGF levels can be measured and are above the assay background in all matrices .

No interference with Neuropilin-1

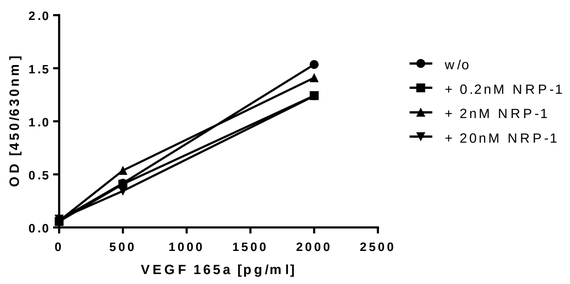

Fig. 5: Neuropilin-1 does not interfere with VEGF detection. Recombinant VEGF 165a was incubated with Neuropilin-1 co-receptor at physiological concentrations (2nM) as well as at lower and higher concentrations. Detection of VEGF was possible in each case, indicating that both states, free VEGF and Neuropilin-1 bound VEGF, are quantified by this ELISA.

Sample Stability

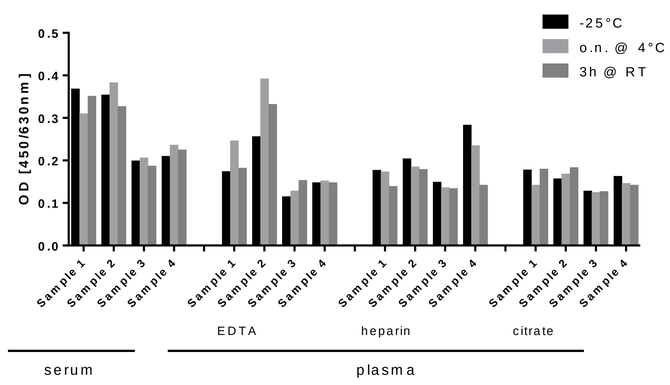

Fig. 6: Stable VEGF in all sample matrices. Stability of endogenous VEGF in different blood preparations was determined at room temperature (3h) and at 4° C (over night) and compared to frozen samples. A deviation of max. 20% between measured values was observed, thus endogenous VEGF proves to be stable in all matrices.

LITERATURE

Hu K, Olsen BR. The roles of vascular endothelial growth factor in bone repair and regeneration. Bone. 2016; 91:3 0–8.

CONTACT

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