Multiplexed Biomarker Determination

U. Zirrgiebel, D. Ziegler and C. Schächtele
ProQinase GmbH, Tumor Biology Center, Freiburg, Germany.

Introduction

Today several new technologies appear on the market that allow the parallel determination of biomarkers in one assay. This report describes the validation of a 4-plex biomarker assay using the electrochemiluminescence reaction developed by MesoScaleDiscovery (MSD, USA). Measurements on the MSD platform are compared with values obtained using the well established Quantikine® Immunoassay kits from R&D (#DVE00 for VEGF and #HSFB75 for high sensitivity bFGF).

Custom coated plates made available from MSD enable the parallel quantification of VEGF, bFGF, sFLT-1 and PlGF. This is accomplished by spotting 4 specific capture antibodies per well in a defined spatial pattern. After binding of the proteins present in the sample, detection is performed using a mix of 4 respective detection antibodies. These antibodies are specifically tagged to emit light after electrochemical stimulation (Reader: MSD Sector 2400). Both ELISA kits from R&D and the MSD system translate the signal into protein concentration by comparing the signal intensity of unknown samples with the signal obtained of known amounts of recombinant standard protein.

Direct data comparison between R&D ELISA kits and the MSD system focussed on VEGF and bFGF. In a second set of experiments we evaluated the recovery of defined amounts of recombinant proteins on the background of a regular plasma sample.

MSD Assay principle

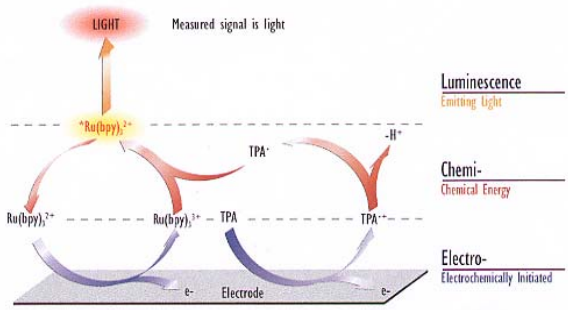
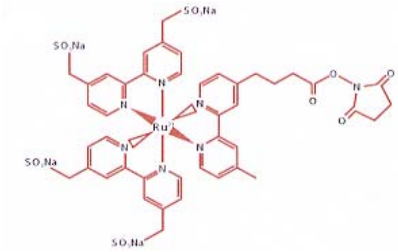
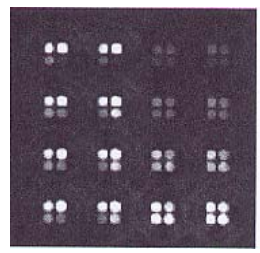


Figure 1: The procedure resembles the well known sandwich ELISA technique, but is based on detection of electrochemiluminescence (ECL). Capture antibodies are immobilized on the surface of the assay plates. The detection antibody is labeled with $Ru(bpy)_3^{2+}$. Upon application of an electric current, the antibody-bound $Ru(bpy)_3^{2+}$-ligand undergoes an oxidation-reduction cycle in the presence of a coreactant tripropylamine (TPA) and emits light. Signal is only generated when the $Ru(bpy)_3^{2+}$-label is in close proximity to the electrode, thus discriminating the bound label from unbound and enabling a no wash, homogeneous format. Detected light intensity is proportional to the amount of captured protein.

MSD SULFO-TAG™ label



Ruthenium (II) tris-bipyridine-(4-methyl-sulfone) NHS ester



4plex ECL signal detected with a CCD camera (Reader: MSD Sector 2400)

Multiplex plate to determine 4 different soluble biomarkers in parallel

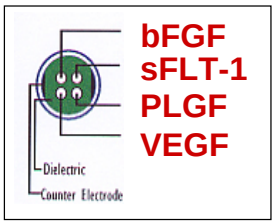


Figure 2: Custom coated plates made available from MSD enable the parallel quantification of VEGF, bFGF, sFLT-1 and PlGF. This is accomplished by spotting 4 specific capture antibodies per well in a defined spatial pattern. After binding of the proteins present in the sample, detection is performed using a mix of 4 respective detection antibodies. These antibodies are specifically tagged to emit light after electrochemical stimulation (Reader: MSD Sector 2400).

Calibration curves with rec. proteins

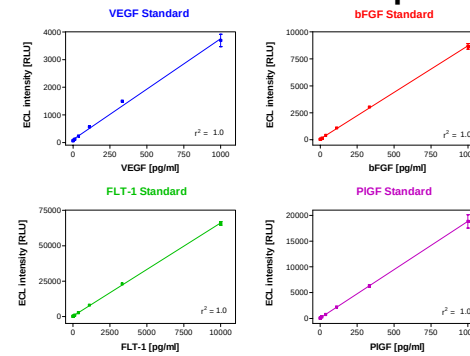


Figure 3: The signal is translated into protein concentration by comparing the signal intensity of unknown samples with the signal obtained of known amounts of recombinant standard protein. Standard curves for all 4 biomarker proteins are determined in parallel on one 4plex biomarker plate. Values were generated in duplicates and linear regression was performed using GraphPad Prism 3.03. In all cases the goodness of fit (r^2) was 1.0.

Correlation of MSD with R&D ELISA data

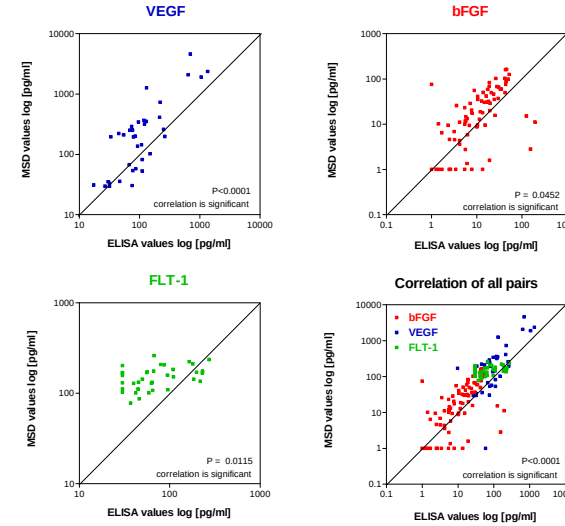


Figure 4: Correlation of biomarker concentrations determined either with the 3 R&D ELISA kits and the MSD 4plex biomarker plate in parallel. In all cases the correlation is significant.

Recovery of spiked samples

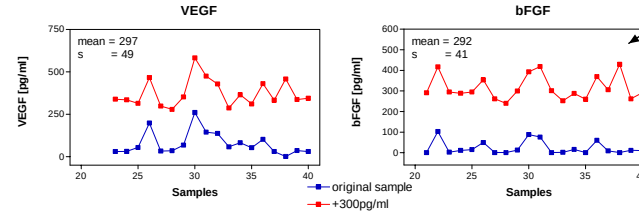


Figure 5: Original plasma samples have been spiked with known concentrations of VEGF and bFGF. The resulting concentrations demontstrated that the rate of recovery of the respective recombinant protein on the background of total plasma proteins is very high (99% for VEGF and 97.3% for bFGF).

Conclusion

Calibration curves of both systems are linear with a very limited degree of variability. Direct comparison of VEGF and bFGF levels of patient samples determined with R&D ELISAs and the MSD system shows a highly significant level of correlation (P<0.0001). The recovery of recombinant VEGF and bFGF in spiked samples determined with the MSD system is satisfactorily and demonstrates the expected additive behaviour.

Furthermore, besides the advantage of determining 4 parameters in parallel, the MSD system works with much smaller sample volumes. Another plus is the ease of handling of the MSD platform, which markedly reduces assay duration. By introducing this new technology ProQinase guarantees that our customers will benefit accordingly.