

## **appendix e-2**

### *Analysis of soluble interleukin-2 receptor as cerebrospinal fluid biomarker for neurosarcoidosis*

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#### **Calculation of soluble interleukin-2 receptor values in U/ml**

Soluble interleukin-2 receptor (sIL-2R) was determined in cerebrospinal fluid (CSF) and serum samples using IMMULITE™ semi-automatic chemiluminescent immunoassays (Siemens Healthcare GmbH, Erlangen, Germany). Results of these measurements are reported by the IMMULITE™ analyzer in counts per seconds (CPS) and U/ml. The IMMULITE™ analyzer is programmed to consider sIL-2R levels of 50 U/ml as the lowest detectable value and reports levels below 50 U/ml as <50 U/ml. As non-inflammatory CSF samples contain only low levels of sIL-2R, sIL-2R values were reported as <50 U/ml in a number of those CSF samples. However, the analytical sensitivity of the IMMULITE™ sIL-2R assay is 5 U/ml. We therefore decided to calculate sIL-2R levels in U/ml also in CSF samples with sIL-2R values <50 U/ml by creating a standard curve using CPS and U/ml data from all CSF samples from which CPS were available and which had sIL-2R concentrations >50 U/ml. To this end, we plotted logarithmized (log 10) values of CSF sIL-2R in CPS and in U/ml in a XY-diagram and performed a linear regression analysis, yielding the following formula:

$$\text{CSF sIL-2R (U/ml)} = (\text{CSF sIL-2R [CPS]} - 4.4133)/0.8897$$

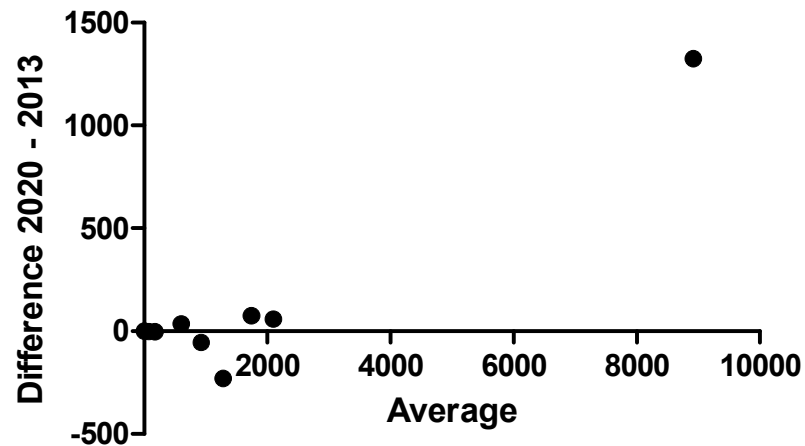
Applying this formula, CSF sIL-2R values in U/ml were then calculated from CSF sIL-2R CPS values for all CSF samples analyzed in this work. There was a strong correlation ( $r^2 = 0.99$ ) between CSF sIL-2R values in U/ml reported by the IMMULITE™ analyzer and the sIL-2R values (U/ml) calculated by the above formula.

The upper limit of the calibration range of the IMMULITE™ sIL-2R assay is 7500 U/ml. In 4 samples from patients with neurotuberculosis, CSF sIL-2R levels were reported by the IMMULITE™ analyzer as >7500 U/ml and thus exceeded the linear range of the standard curve. We therefore did not include the CSF sIL-2R data from these 4 samples in the analysis.

## Stability of sIL-2R in CSF and serum samples stored at -20°C for 7 years

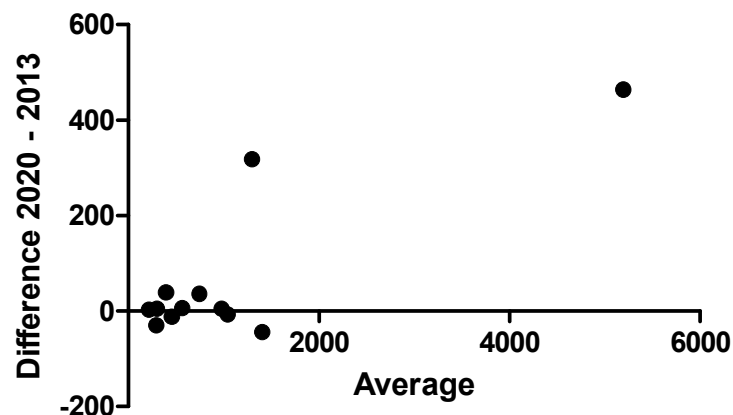
**A**

**CSF sIL2R measured in 2020 and 2013 (U/ml)**



**B**

**serum sIL2R measured in 2020 and 2013 (U/ml)**



Bland-Altman plots of sIL-2R measurements performed in the same CSF (A; n = 12) and serum (B; n = 12) samples in 2013 and 2020 after approximately 7 years of storage at -20°C. The plots show the average of both measurements vs. the difference between measurements performed in 2020 and 2013 in U/ml. Note that in 4 CSF samples sIL-2R values were below the cut-off of 50 U/ml in 2013 as well as in 2020 and these values were set as 0 for the purposes of this evaluation.